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DETERMINATION OF SEX IN ELODEA

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(WITH PLATES XXIII–XXVII AND EIGHT FIGURES)

In studying the reduction mitoses in *Elodea gigantea* (38), some differentiation was found among its chromosomes, but only male plants were available; consequently, it seemed desirable to investigate our native species, *E. canadensis*, in which both male and female plants are abundant.

My previous paper described the work already done on the determination of sex in plants, beginning with STRASBURGER (44), who reviewed the theory that sex is determined by the surrounding conditions; the cytological work published by DARLING (10) on *Acer negundo*, who claimed that he found an unequal distribution of chromatin in the nuclei resulting from the homotypic division; the work of MOTTIER (33), who studied the same plant but obtained different results; and the experimental and cytological investigation of ALLEN (1) on *Sphaerocarpus*, with its direct relation to the experimental work of C. and R. DOUIN (15) on the same genus; those of the MARCHALS' (28, 29) on mosses; CORRENS (9) on *Bryonia dioica* and *B. alba;* NOLL (34) on *Cannabis;* and STRASBURGER (45) on *Mercurialis annua*, *Melandrium album*, and others.

Material and methods

The material used in this investigation was collected in the early part of June 1922, from Wolf Lake, about fourteen miles from Chi-

cago, where STRASBURGER's material was collected. Ten sets were fixed in the field on different days and at different times of the day; and four sets were pickled in the greenhouse, where a quantity of the plant was kept. In addition, some material collected in August 1921 was used.

The fixing reagent was 1 per cent chromo-acetic acid, with about ten drops of 1 per cent osmic acid to 50 cc. of the solution. The material was imbedded in 52° C. paraffin, and cut 3–4 μ thick, with some 10–15 μ thick, and stained in Haidenhain's iron-alum haematoxylin with methyl orange.

Heterotypic prophase to synizesis

The reduction divisions in *E. canadensis* agree in almost every respect with those of *E. gigantea*, as described in my former paper (**38**), but all the structures are smaller. The pollen mother cell following the last mitosis of the archesporial region is distinctly polygonal in outline in cross and longitudinal sections of the anther. Its cytoplasm is dense, and more or less uniformly granular. The nucleus may be in the center of the cell or very close to one side. Its structure consists of a chromatin network or mesh, with nodelike structures or beadlike enlargements in the intersections of the chromatin threads. Sometimes these fine chromatin threads and nodes are observed in pairs. According to Miss DIGBY (**13**), this is the first indication of the reassociation of the chromosome halves (threads) which separated during the preceding telophase. Fig. 1 illustrates the earliest definite prophase of the first reduction division. The nucleolus may be vacuolated or not, and may be in the center or near to the periphery of the nucleus.

As the nucleus enlarges the reticulum loses its reticular character, the chromatin nodes become larger and closer together, and the threads become thicker. This is perhaps due to the fact that the chromatin threads are gradually contracting and pairing. I was unable to find a place at this stage where the chromatin threads are not doubled. Whether or not this pairing is due to mere "chance parallelisms," as claimed by CLELAND (**8**), the results in *Elodea* seem to agree with the findings of Miss DIGBY (**13**) in *Osmunda*. Fig. 2 shows the gradual pairing of the threads, and in fig. 3 this

pairing has become more evident. From this stage the nuclear contents are slowly withdrawn from the nuclear periphery, which indicates that synizesis is about to take place. In figs. 3 and 4 the appearance of a clear space on one or two sides of the nucleus is represented. This period of development of the mother cell is brought about by the contraction of the nuclear contents (chromatin threads) and by the enlargement of the nuclear cavity (38).

SYNIZESIS.—As the nucleus advances into synizesis, the pairing and contraction continue, until at last a more or less compact ball of chromatin at one side of the nucleus is formed. In the majority of cases, but not always, the synizesic knot lies on that side of the nucleus which is nearest the periphery of the cell. This is the most critical stage in the development of the mother cell, and it remains in this condition for a comparatively long period. In *Zamia* this stage lasts for several days, the structure of the knot being so tight that sometimes it looks like a granular precipitate from a chemical reaction entangled in a mass of threads. In most cases one or two loops of threads project from the knot. A careful examination of thin tangential sections of the chromatin ball, however, shows that the chromatin granules were brought close together by the contraction of the threads. In many cases these chromatin nodes or granules run in rows side by side. Fig. 5 represents a typical knot in which some of the chromatin threads are projecting in loops from the mass. As this process advances, the spireme becomes more and more distinct and uniform. Miss DIGBY believes that during this period of the life of the mother cell, the association of threads in pairs, prepared during the presynaptic stages, is consummated. All the evidences in *Elodea* seem to support this theory. The spireme threads during the later part of synizesis show a double character all the way through, and become very much thicker than before. Up to this stage no material change is observed in the shape and size of the nucleolus, except that its vacuoles are more pronounced, and occasionally protuberances or budlike structures are noticed coming out from its surface.

OPEN SPIREME.—Soon after the maximum synizesic contraction is attained, the knot loosens its structure gradually, and the closely doubled threads begin to extend slowly throughout the nuclear

cavity, until they are more or less uniformly distributed (figs. 6–8). Meanwhile the spireme becomes thicker, and appears as a homogeneous, smooth, single, and continuous filament, although in some places free ends may be observed. A critical examination of the spireme at this stage, however, reveals the fact that in many cases the chromatin nodes are found in two close and compact rows, which appear single at first glance.

SECOND CONTRACTION.—Immediately after the distribution of the spireme in the nuclear cavity, loops are formed (fig. 9). These loops are especially characteristic before the partial segmentation and second contraction take place (figs. 10, 11). Shortly after the numerous loops are thrown and spread through the nuclear vacuole, the spireme passes into the condition known as second contraction, and during this period the chromatin filament becomes thicker and thicker, until its double nature can no longer be detected. I believe that this is the most critical part of the nuclear development. The loops become rearranged, their sides begin to approach one another, and they gradually become detached. The segments of the spireme become entangled, and it is almost impossible to determine the nature and number of the individual pieces. Fig. 12 shows a stage preparatory to second contraction, in which the two ends of some of the loops have twisted around each other. Figs. 13 and 14 represent the character of the second contraction in *Elodea*. Occasionally at this stage a constriction is observed at the distal end of the loop, which indicates that each side of the loop represents a univalent chromosome. During the later part of the second contraction, and before the individual segments are thrown to the periphery, the pollen mother cells, which during the resting period and up to the later stage of synizesis were packed closely, are beginning to round off and separate from one another.

Diakinesis

The knot formed by the second contraction does not seem to be of long duration, judging from the scarcity of this stage, but soon loosens, and the young bivalent chromosomes are thrown toward the periphery of the nucleus. This is indicated in fig. 15, in which the young heterotypic chromosomes are just coming out from the

second contraction. Some of them are in the form of 8's, due to the twisting about each other of the parallel sides of the loops; some still have the *U* shape; and others are composed of two straight or slightly curved rods lying side by side and sometimes slightly twisted about each other; or the two segments adhere at one end, taking a more or less *V* shape. Closely following the stage shown in fig. 15 is that of a complete distribution of the partially condensed bivalent chromosomes shown in fig. 16, in which some of the chromosomes are clearly marked out, while others are still in the process of organization. Beginning at this stage, a critical study was made of the differentiation among chromosomes. The numerous counts made agreed remarkably with the number of bivalent chromosomes in *Elodea gigantea;* and even the characteristic unequal pair and the large or giant chromosome described in my earlier paper (**38**) were found. In text figs. 1 and 2 are two groups of the young heterotypic chromosomes, in which it is easy to distinguish the differences in size. Fig. 17 represents a later stage of diakinesis. This is one of the most favorable stages for the observation of the unequal pair of chromosomes, when one is fortunate enough to get the proper orientation, for sometimes the chromosomes are close to the periphery and point upward, both of which conditions make it difficult to recognize the exact outline of the two members of the pair.

It is important to notice that during the later period of the second contraction, up to the later period of diakinesis, the nucleus becomes more and more vacuolated and seems to be thin and empty. The cytoplasm near the nuclear membrane in the meantime becomes denser, making the nuclear wall seem thick and irregular in outline. This is the first indication that the spindle fibers are about to appear. At the time the spindle fibers appear, the nuclear membrane and the nucleolus disappear simultaneously. This has led to the suggestion that both the membrane and the nucleolus have been used in the formation of the spindles (**38**), or that in some way they contribute to the formation of spindle fibers, as has been emphasized by HARPER (**18**), GATES (**16**), DAVIS (**12**), and Miss DIGBY (**13**). On the other hand, LAWSON (**26**) believes that the nuclear membrane does not break down and is not used up in spindle formation, but

that it becomes applied to, and completely envelops the surface of each chromosome. I have come to the conclusion, however, that not all the material for the spindle fibers is stored in the nucleolus and nuclear membrane, nor do these structures disappear completely; but their material is deposited in the cytoplasm, particularly on the sides of the nucleus toward the two poles, during the later period of the telophase. They reappear at the time the nuclear membrane is broken down, or even before the disorganization of the nuclear wall. Their structure in the early period of their formation shows that they consist of rather coarse threads with uniform nodal enlargements which look like a string of beads. When close together they give the general appearance of the structure of the cytoplasm. The beads disappear, however, as soon as the spindle fibers are stretched toward the two poles. Whether this beadlike structure is due to nucleolar exudation or disintegration I have not been able to determine. Fig. 18 is a longitudinal section of the nucleus, showing the young spindle fibers emerging from at least four parts of the nucleus. Fig. 19 is similar, except that it is cut crosswise. At this stage the bivalent chromosomes are greatly condensed and well distributed throughout the nuclear vacuole. The fission between the two monovalent chromosomes that composes the bivalent chromosome is practically indistinguishable. Owing to the irregular position of the chromosomes, it is very hard to detect the uneven pair, but the giant one is evident.

Heterotypic mitosis

While the spindle fibers are developing and the nucleolus and nuclear membrane are disappearing, the heterotypic chromosomes undergo a process of condensation, until they are reduced to about one-half or less of their original size shown in the late diakinesis stage. This stage is represented in fig. 20, in which the fibers are stretching themselves and have lost their beaded character, while the chromosomes are moving toward the equatorial plate. Detailed drawings of the chromosomes of this stage are shown in the text figs. 3 and 4. An oblique polar view of the chromosomes arranged in the equatorial plate is represented in fig. 21. They exhibit the same differences in size and shape and number as was observed

FIGS. 1–8.—Eight chromosome groups of *Elodea canadensis:* figs. 1 and 2 taken from early stage of diakinesis, with 24 bivalent chromosomes each; figs. 3 and 4 from late diakinesis stage or early metaphase; figs. 5 and 6 from metaphase stage, also with 24 bivalent chromosomes; fig. 7 from stem tip of male sporophyte, with 48 univalent chromosomes; and fig. 8 from stem tip of female sporophyte, with same number of chromosomes as male.

in *Elodea gigantea*. The large chromosome is evident as well as the unequal pair.

Soon after the 24 bivalent chromosomes become arranged in a definite plate, at which time they appear more or less globular and oblong ovoid, the fibers begin to pull apart gradually the two halves of each bivalent chromosome. A typical side view of the metaphase of the heterotypic mitosis is represented in fig. 22, while fig. 23 shows late metaphase or early anaphase. In these two stages the giant chromosome is obviously splitting into two equal halves; occasionally it lags somewhat. On the other hand, the chromosome with unequal members is characterized by dividing earlier than the others. Frequently the small member is observed moving ahead, so that it is distinctly in advance of the rest of the chromosomes of its group. The point of attachment of the spindle fibers does not seem to be definite. Generally it is at the end, but sometimes it is a little toward the middle (text figs. 5, 6). The dual nature of the daughter chromosomes is practically unrecognizable, but in some instances may be traced more or less at their tips.

Two stages of the anaphase, one following the other, are seen in figs. 24 and 25, in which most of the daughter chromosomes (somatic chromosomes) are *V*-shaped, with the apex of the *V* attached to the spindle. In both stages the giant chromosomes and the small member of the uneven pair are easily seen. The double nature of the haploid chromosomes is more or less distinguishable at their tips. These pass entire to the pole, where they form a little cluster in the anastomosing condition (fig. 26). Then the nuclear membrane, followed later by the nucleolus, begins to appear, and the wall between the daughter cells is gradually formed by the thickening of the middle part of the spindles. The daughter nuclei enlarge immediately and assume an ovoid form. They are characterized by the fact that they do not reach a true resting condition, but soon prepare for the second division.

Homotypic mitosis

The life of the nuclear membrane of the daughter nuclei, resulting from the heterotypic mitosis, is of short duration, because the second meiotic division follows quickly. As the two nuclear

membranes break down and the two nucleoli disappear, the two homotypic spindles appear simultaneously. These may lie parallel or at right angles to each other, or sometimes in a *V*-shaped position (figs. 29, 30). Fig. 28 shows two groups of chromosomes in the polar view of the two daughter nuclei arranged in the equatorial plate. Even in this stage the large chromosome and the small one from the unequal pair are noticeable. In this second division all the daughter chromosomes are split equally, and, as usual, the small chromosome divides earlier than the others (figs. 29, 30). Fig. 31 is at about the same stage as fig. 30, and illustrates the other arrangement of the two homotypic spindles. The four granddaughter nuclei are formed about as the daughter nuclei in the first mitosis. Two of them, however, are slightly smaller than the other two (fig. 32). The nuclei grow rapidly and pass quickly into resting stage. At this stage the difference in size of the nuclei is almost imperceptible. Fig. 33 is an older stage in which the four nuclei are almost alike. To learn whether the mature pollen grains are affected by such differences as were observed in the size of the four young granddaughter nuclei, I made the following measurements of the microspores collected at the time they were shed:

First pair	Second pair
Average diameters	Average diameters
102.73 $\mu \times$ 97.35 μ	94.11 $\mu \times$ 85.65 μ
101.88 $\mu \times$ 97.36 μ	93.77 $\mu \times$ 85.67 μ

Each of these averages is calculated from twenty-five measurements. It is evident that two of the resulting microspores are slightly larger than the other two. Although this difference is rather small, it is very significant in its relation to the unequal distribution of the chromatin, due to the small chromosome from the uneven pair in the heterotypic mitosis.

It is interesting to notice that the four microspores at an early stage are within the wall of the mother cell, but soon the wall disappears, so that they are no longer inclosed. At this stage the young tetrads enter upon a period of rapid enlargement. One peculiarity of the tetrad is that its four members do not fall apart, but remain attached and are ultimately shed from the sporangium still firmly joined together. They have a strongly cutinized exine and a well developed entine.

Somatic mitosis of formation of tube and generative nuclei

TELOPHASE TO RESTING STAGE.—Soon after the chromosomes in the homotypic mitosis have reached the poles and have formed a more or less compact knot, the nuclear membrane begins to appear. At this stage the chromosomes seem to lose temporarily their individuality, but as soon as the rapid growth of the nucleus proceeds, their individual boundaries again become visible. They appear much elongated, lying parallel to each other across the nuclear cavity, sometimes with much regularity, with their sides cohering at various points. While the enlargement of the nucleus is in progress the nucleoli appear suddenly, and the chromosome bands become vacuolated (figs. 34, 35). GRÉGOIRE (17), in his work on *Allium*, has shown that each chromosome shows two parallel lines, composed of threads or granules. In this somatic mitosis of *Elodea* it seems that the same process is taking place. The splitting of each chromosome into two halves is evident. This is in accord also with the idea proposed by Miss DIGBY and others. The vacuolization and splitting continue until a beaded chromatin framework or mesh is formed, which is the stage commonly known as "resting." This condition in the pollen nucleus seems to persist for a comparatively short period, and soon the nuclear contents begin to reorganize by the pairing of the chromatin threads (fig. 36).

CHROMOSOME FORMATION.—The pairing of the chromatin threads in the pollen nucleus of *Elodea* seems to occur with much regularity. I have examined a great number of them at this stage, and was unable to find a single one in which the young spireme was not doubled. The chromatin granules appear also with regularity in two close parallel rows (figs. 38, 39). As the nucleus enlarges, the spireme becomes more distinct and thicker and apparently loses its double nature. Once in a while one can find, here and there, some free ends. This perhaps is due in some places to the sectioning. The granules also appear single, but double the size of those in the resting stage (fig. 40). At this stage the spireme seems to be almost uniform in diameter and almost straight, radiating in different directions across the nuclear cavity. The first sign to be noticed before segmentation of the spireme is that it becomes very irregular

in diameter, that is, in certain places it is very thick and wavy, but thin in the other places (fig. 41). Closely following this stage is that shown in fig. 42, in which the individual segments are distinctly seen separating from each other. Fig. 43 shows the partial condensation of the chromosomes which have become individualized.

METAPHASE TO TELOPHASE.—When the maximum condensation has been attained by the chromosomes, and when the nuclear membrane and the nucleolus have disappeared, the chromosomes arrange themselves in the usual manner in the equatorial plate. Figs. 44 and 45 are two polar views taken from the same tetrad, in which it is easy to count 24 chromosomes in each group. This is the best stage in the formation of the pollen grain for counting the number of chromosomes, for they are not so crowded together. The difference in size of the chromosomes is easily seen. In fig. 44 the large chromosome (*L*) and the small one (*m*) are evident; while in fig. 45 only the large one is present. It is easy to conclude that they are not pairmates.

Typical side views of the metaphase stage are represented in figs. 46–49. It was fortunate, after examining many tetrads, to get these four nuclei in division and with proper orientation, so that I was able to trace and identify the large (*L*) and small (*m*) chromosomes. Judging from the presence of these two types of chromosomes, it is evident that figs. 46 and 47 are from the one nucleus, and figs. 48 and 49 from the other. From the metaphase the chromosomes pass to their respective poles in the usual way. Late anaphase is shown in figs. 50 and 51. Both nuclei are from the same tetrad. In fig. 50 the giant (*L*) chromosome is indicated, while in fig. 51 both the large (*L*) and small (*m*) one can be identified. Closely following this figure is the stage shown in fig. 52, with the appearance of the nuclear membrane, the resolution of the chromosomes in the usual fashion, and the beginning of the formation of the temporary wall between the generative cell and tube cell. A more advanced stage is shown in fig. 53, in which the thick wall (*w*) of the pollen, the generative cell (*g*), and the tube nucleus (*tn*) are passing into the resting stage. The spindle fibers remain until this stage but soon resolve themselves into reticulate cytoplasm.

Chromosomes of male and female sporophytes

A careful examination of the chromosomes of the male and female sporophytes showed that there are 48 somatic chromosomes in each of their dividing cells in the meristematic tissue. On account of the great number of chromosomes it was difficult to count them, and to get a proper orientation for the examination of their differences in size. After examining a number of the root and branch tips, however, I found that in most cases there were 48 somatic chromosomes in both male and female. In some instances the numbers 46 and 47 were met, but I accounted for this variation by their frequent overlapping. Moreover, since 48 is double the number of the bivalent chromosomes in the meiotic mitosis, I believe it is the exact number for the somatic chromosomes.

In examining critically the size of the chromosomes of the male, among the 48 of them, two are extraordinarily large, exceeding in length and in width the other chromosomes, while one of them is very small. The latter is not always easy to find except where favorable orientation and good scattering of the chromosomes are obtained. Evidently, in the reduction division the two large chromosomes unite with each other, and the small one unites with a chromosome of about the same size as the rest, and thus the unequal pair is formed. Fig. 54 represents a typical group of somatic chromosomes from a branch tip of the male sporophyte. On the other hand, in the female sporophyte, in most of the cases, out of the 48 chromosomes two of them are as large as those found in the male sporophyte, and the rest have approximately the same size (figs. 55, 56). Fig. 55 is taken from a section of a very young leaf near the tip of the branch, in which each of the large chromosomes has divided into two halves; and fig. 56 is also taken from a branch tip, showing the two large chromosomes corresponding to those found in the male sporophyte.

Relation of chromosomes to sex

Elodea canadensis shows an extremely close agreement in the general history of the chromosome group, and especially in the behavior of the unequal pair and the giant chromosome with the condition found in *E. gigantea*. In the prophase of the somatic mi-

tosis of the male and female sporophytes, I found in both cases 48 univalent chromosomes. In the male, out of the 48, two are extraordinarily large, exceeding the other chromosomes both in length and width, and one is very small (fig. 54). In the female plant there are two large chromosomes, corresponding in size with those in the male, and 46 chromosomes approximately alike (figs. 55, 56). On the other hand, in the maturation division of the male, I found 24 bivalent chromosomes, which is evidently half of the number in the somatic. Out of these 24, one exceeds the other chromosomes in length and width, and another is characteristic because of the unequal length of its component elements. It is clear that the two large chromosomes of the somatic nuclei have united, and the small one has paired with a chromosome of about the same size as the chromosomes, to which the term autosome is applied, resulting in an uneven pair. These separate from each other in the first division, in which one of the daughter nuclei receives one giant chromosome, the larger member of the unequal pair, and 22 autosomes; while the other daughter nucleus receives one giant chromosome and a very small one (from the unequal pair), and 22 autosomes. In the second maturation division each chromosome of the daughter nuclei divides equally, and thus two of the four resulting daughter nuclei receive one giant and one small chromosome and 22 autosomes; while the other two receive one giant chromosome, the larger member of the unequal pair, and 22 autosomes. It is evident, therefore, that there is an unequal distribution of chromatin as in the other species of *Elodea*. This unequal distribution of the chromatin affects in some way the size of the resulting four nuclei during their early stages. Two of them are slightly smaller than the other two, and by re-examining the slides of *E. gigantea* I found the same differences, and they do not, as stated in my previous paper (**38**), look alike. The resulting pollen grains show also slight differences in size, two of them being smaller than the other two, as previously demonstrated.

The presence of the unequal pair in the meiotic division, and the small chromosome in somatic mitosis in the male sporophyte, show that the unequal pair has a direct bearing upon the determination of sex. The behavior of the two members of the unequal

pair agrees remarkably with that of the *Y* and *X* chromosomes in *Drosophila* (STEVENS **43,** MORGAN **32**, METZ **30,** and others); the heterochromosomes or idiochromosomes in *Lygaeus* (WILSON **48, 49**); *Euschistus* (MONTGOMERY **31**); and other insects. It also agrees with the *X* and *Y* chromosomes found in *Sphaerocarpus*, a dioecious bryophyte, by ALLEN (**1**). In view of this similarity, undoubtedly the chromosomes in the unequal pair bear sex characters, as in the insects and *Sphaerocarpus*. For this reason the small chromosome is marked *m*, indicating its male character, and its mate *f*, indicating its female character. The large, or giant, chromosome is marked *L*. This giant chromosome may have some special function in heredity, but so far as my observations are concerned, it behaves like an ordinary autosome.

Discussion

In regard to the question of sex determination in *Elodea* and its relation to the accumulated evidences found in animals as well as in plants, I have very little to add to what was given in my previous paper. The unequal pair evidently plays a very important rôle in the distribution of the chromatin to the four microspores. This unequal distribution of chromatin to the microspores is doubtless responsible for the 1:1 ratio of the sex distribution in plants, as has been observed in animals. WILSON (**48, 49**), however, believes these quantitative differences cannot always be considered as primary sex determining factors, for he found that such a view is inapplicable to cases like *Nezara*, *Oncopeltus*, or *Metapodius*. In most of the cases studied in animals, however, the unequal distribution of the chromatin prevails. So, in the case of *Elodea*, I firmly believe that the *m* and *f* chromosomes function in the determination of sex.

In my previous paper I have quoted the five classes of the much studied cases in animals published by WILSON (**48, 49**), in his comparative review of the types of sexual differences of the chromosome groups. In his classification, despite the apparent diversity of the types, all are in accord with the principle that the spermatozoa are of two kinds, equal in number, respectively male producing and female producing. The case of *Elodea*, as already indicated, agrees remarkably with those in the second group, and at the same time it is in accord with the principle.

The question of experimental evidences in sex determination in plants has been discussed in my previous paper, which emphasized the work of the MARCHALS (**28, 29**) on mosses; DOUIN (**14**) and ALLEN (**1**) on *Sphaerocarpus;* CORRENS (**9**) on *Bryonia dioica* and *B. alba;* NOLL (**34**) on *Cannabis;* STRASBURGER (**45**) on *Mercurialis annua* and *Melandrium album;* and others. In all these investigations it was found that the sex ratio is about 1:1, or half male and half female. By changing the external conditions, they claim that they were not able to change that ratio.

The monoecious plants are quite abundant in the plant world, but the dioecious ones are also many. The important question is whether the dioecious plants have been derived from the monoecious ones. Lately some strong evidences that the dioecious plants have been derived from the monoecious plants have been published. STRASBURGER (**45**), in a general discussion of his investigations on *Elodea*, *Mercurialis*, and other plants, has summarized the situation in plants, the report being well translated by SHARP (**42**). In my previous paper I have given ALLEN's and SHARP's views in regard to the separation of sex factors in dioecious seed plants; and also COLLIN's (**6, 7**) results in his experiments on *Funaria hygrometrica*, a dioecious moss, from which he was able to produce a monoecious plant.

Important results have come to light in the last few years, bearing upon the problem of experimental alteration of the sex ratio. Although we know that in most animals the sex ratio runs approximately 1:1, occasionally some deviations have been observed, which have led to some experimentation. Among the important results obtained in animals are those of HERTWIG (**19, 20**) and KUSCHAKEWISCH (**25**). Under normal condition the eggs of a frog give rise to male and female individuals in equal numbers; but by allowing the eggs, before fertilization, to take up an abnormal amount of water, the number of males was considerably increased, sometimes even to 100 per cent. On the other hand, Miss KING (**21, 22, 23, 24**), by lowering the water content of toad eggs, was able to increase the proportion of females. Similar results have been obtained with *Dinophilus* (MALSEN **27**) and *Hydatina senta* (WHITNEY **46, 47**).

In plants a number of experiments of similar nature have given more or less similar results. According to SHARP (**42**), however, these are less conclusive than those in animals, for the reason that most of the experiments have been carried out with angiosperms, in which intersexes are common. Among the plants that have been worked out, and that showed intersexes, may be cited the following: *Cannabis sativa* (PRITCHARD **36**); *Myrica Gale* (DAVEY and GIBSON **11**); *Arisaema dracontium* (SCHAFFNER **41**); *Cannabis sativa*, *Salix amygdaloides*, and *Morus alba* (SCHAFFNER **39, 40**); *Plantago lanceolata* (BARTLETT **3**; CORRENS **9**); *Mercurialis annua* (YAMPOLSKY **51, 52**). So far as *Elodea* is concerned, only one case, reported by HITCHCOCK, showed stamens and carpel in the same flower.

In view of the evidences obtained by the morphologists and physiologists, two theories have been proposed, both with strong supporters. One theory claims that sex is determined and controlled by the presence of the accessory or heterochromosomes; while the other claims that sex is determined and controlled by the environmental factors. It is interesting to notice, however, that those who belong to the morphological side confine their studies practically to that side; and that those who take the physiological view, pay little or no attention to internal structure. I agree with the statement of Professor SHARP (**42**) in his discussion of sex, that "It is beyond question that the two manifestations of sexual differentiation, the physiological and the morphological, are both of importance and cannot entirely be irreconcilable: our task is to determine their relative significance and to discover the nature and degree of their mutual independence." Any experimental studies should be accompanied with a critical cytological investigation. The effect of the environmental condition should not be looked upon from the external character of the individual only, but also in its effect upon the structure of the cell. One apparently may get some changes in form, but in reality the changes may be produced by changes in the chromatin.

Summary

1. The resting nucleus of *Elodea canadensis* consists of a chromatin network or mesh with chromatin beadlike structures in the intersections of the chromatin threads.

2. Before synizesis there is a parallelism of the threads and beads. As the nucleus is in progress of enlargement, the reticulum loses its reticular character, and the pairing of the threads becomes more distinct.

3. The first indication that synizesis is about to take place is the appearance of a clear space on one or two sides of the nucleus, the thickening of the chromatin threads, and the enlargement of the chromatin nodes.

4. The phenomenon known as synizesis consists in the rapid growth of the nucleus and especially in the contraction of the nuclear contents into a more or less compact mass, which may or may not include the nucleolus. This stage lasts for a long period.

5. After synizesis the knot loosens its structure, and the closely doubled threads (spireme) begin to extend gradually throughout the nuclear cavity until it is more or less uniformly distributed.

6. After the distribution of the spireme in the nuclear cavity, loops are formed, after which the spireme passes into the second contraction, and then gradually becomes segmented.

7. The two ends of each of the loops, as they emerge from the second contraction, approach each other and sometimes twist about each other. These consist of two univalent chromosomes, arranged end to end. The univalent chromosomes in *Elodea*, therefore, are arranged telosynaptically.

8. As the 24 bivalent chromosomes emerge from the second contraction, they undergo a considerable condensation, until they acquire a more or less definite form. At this stage occasionally the *m* and *f* chromosomes are evident.

9. The nucleolus and the nuclear membrane disappear simultaneously, while the bivalent chromosomes are drawn to the equatorial plate and the spindles appear almost at the same time. Each of the bivalent chromosomes is attached to the spindle by one end, and the two constituent elements move apart. The giant chromosome splits lengthwise and forms two large daughter chromo-

somes; while the *m* and *f* constituents of the unequal pair separate from each other. The *m* chromosome goes to one pole, while the *f* chromosome passes to the other.

10. As the daughter chromosomes move toward the poles, they become V-shaped, with the apex attached to the spindle. The longitudinal split of the daughter chromosomes preparatory to the homotypic mitosis appears as they come very close to the poles.

11. The daughter nuclei resulting from the heterotypic division do not pass into a true resting stage, not reaching a true reticulum, but soon reorganize for the homotypic mitosis. As the nuclear membranes and the nucleolus disappear, the two homotypic spindles appear simultaneously. The univalent chromosomes arrange themselves in the equatorial plates and split equally lengthwise.

12. In the second division all the daughter chromosomes divide equally, so that two of the four resulting grand-daughter nuclei have received an equal share of the *m* chromosome, while the other two have received an equal share of the *f* chromosome from the unequal pair. The two that received the halves of the *m* chromosome have less chromatin than the two that received the halves of the *f* chromosome. Moreover, the two of the four resulting microspores that received the smaller amount of chromatin are slightly smaller than the two that received the larger amount.

13. The dual character of the univalent chromosomes becomes distinct from late anaphase to telophase. The fission between the two halves of the chromosomes becomes more evident as the nucleus proceeds to the resting stage.

14. Before the resting stage, a row of vacuoles appears along the fission of the chromosomes and splits them into halves or threads. As the nucleus enlarges, these are stretched and uniformly distributed in the nuclear cavity, in the form of a network or mesh, which becomes beaded.

15. At the resting stage the nuclear contents consist of a fine network of chromatin threads with chromatin enlargements in the intersections of the threads.

16. Soon after a short period of rest, the reticular character of the nucleus becomes disarranged. The chromatin threads with the chromatin nodes are brought together in parallel pairs, until

the spireme is well organized. This becomes thicker and irregular in diameter, and finally is segmented into 24 univalent chromosomes.

17. At the metaphase stage the giant *L* chromosomes, as well as the *m* chromosome, are easily seen. Each of the univalent chromosomes splits equally lengthwise, and the halves of the chromosomes proceed to their respective poles in the usual manner.

18. The somatic chromosomes of the male sporophyte are 48 in number. Out of the 48 univalent chromosomes, two are extraordinarily large, exceeding the other chromosomes in length and width; and one is very small.

19. The female sporophyte contained the same number of chromosomes as the male sporophyte. There are also two large ones that correspond in size to the two large chromosomes in the male, but the rest are approximately alike.

I wish to express my acknowledgment and gratitude to Professor CHARLES J. CHAMBERLAIN for proposing the problem, and also for suggestions during the course of the investigation.

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EXPLANATION OF PLATES XXIII–XXVII

All figures drawn with aid of camera lucida, using a Zeiss 2 mm. N.A. 1.4 oil immersion objective and 18X eye-piece.

PLATE XXIII

FIG. 1.—Typical resting stage of nucleus of pollen mother cell.

FIG. 2.—Older stage, showing chromatin threads gradually approaching each other side by side.

FIG. 3.—Beginning of prophase, in which in upper and lower part can be seen a clear space between nuclear membrane and reticulum.

FIG. 4.—Median section of nucleus, indicating gradual withdrawal of reticulum from nuclear wall, by contraction.

FIG. 5.—More or less complete synizesis, in which nucleolus has not been included in balled-up chromatin network.

FIG. 6.—Early stage of spireme, just emerging from synizesic ball.

FIG. 7.—Later stage in loosening up of spireme, with thread much thicker and well defined, appearing as a homogeneous, single, and continuous filament.

FIG. 8.—Complete hollow spireme with some loops beginning to form.

FIG. 9.—More or less tangential section, indicating characteristic loops preparatory to second contraction.

FIGS. 10, 11.—Partial segmentation before second contraction.

FIG. 12.—Beginning of second contraction, in which ends of loops are twisted about each other.

PLATE XXIV

FIGS. 13, 14.—Two complete second contraction stages.

FIG. 15.—Bivalent chromosomes emerging from second contraction; shape of nucleus becoming ovoid or oblong.

FIGS. 16, 17.—Two successive diakinesis stages, showing about 24 bivalent chromosomes; *L*, large chromosome, and *mf*, unequal pair.

FIG. 18.—Median section, showing multipolar spindle emerging from nuclear wall and radiating across nuclear cavity; spindle at this stage beaded.

FIG. 19.—Another stage similar to that in fig. 18, except that it represents a cross-section of nucleus.

FIG. 20.—Spindle becoming bipolar and losing its beaded character, while bivalent chromosomes are moving toward equatorial plate.

FIG. 21.—Oblique polar view, in which chromosomes are arranged in equatorial plate; *L*, giant chromosome, and *mf*, unequal pair.

FIG. 22.—Complete side view of rather late metaphase, showing early separation of *m* and *f* chromosomes.

FIG. 23.—Early stage of anaphase or late metaphase, in which some of chromosomes are lagging, while *m* chromosome is distinctly ahead.

PLATE XXV

FIG. 24.—Typical side view of anaphase, showing most of daughter chromosomes in V-shape, with apex of V attached to spindle.

FIG. 25.—More advanced heterotypic anaphase: *m* chromosome not easily distinguished at this stage.

FIG. 26.—Early telophase, showing univalent chromosome forming cluster, while nuclear membrane begins to appear.

FIG. 27.—Late telophase, indicating very irregular shape of daughter chromosomes apparently going to resolve, but remaining more or less in this stage for a short period and not passing to true resting; wall between two daughter nuclei becoming distinct by thickening of middle part of spindle.

FIG. 28.—Polar view of daughter chromosomes preparing for second division; even at this stage *L* and *m* chromosomes distinguishable.

FIG. 29.—Side view of homotypic mitosis in metaphase stage; *M* chromosome obviously dividing ahead of rest.

FIGS. 30, 31.—Two different views of homotypic anaphases; in fig. 30, peculiarity of *m* chromosome displayed again.

FIG. 32.—Four young grand-daughter nuclei derived from single microspore mother cell lying in one plane; two decidedly smaller than other two; grand-daughter chromosomes resolving into resting condition.

PLATE XXVI

FIG. 33.—More advanced stage of grand-daughter nuclei, in which all are in prophase; difference in size almost imperceptible.

FIGS. 34, 35.—Two stages of grand-daughter nuclei, showing vacuolization and splitting of chromosomes proceeding to resting stage.

FIG. 36.—Typical median section of resting stage.

FIGS. 37, 38.—Two close stages, indicating parallelism of somatic chromatin threads.

FIG. 39.—More advanced, showing young spireme; double nature noticeable here and there.

FIG. 40.—Typical spireme, appearing single and uniform and coarsely beaded.

FIG. 41.—Spireme contracting and thickening in preparation for segmentation; notice irregularity of diameter.

FIG. 42.—Spireme segmenting into 24 univalent chromosomes.

FIG. 43.—Individual segment shortening and thickening.

PLATE XXVII

FIGS. 44, 45.—Two typical polar views of univalent chromosomes in prophase, taken from same tetrad; one shows *L* and *m* chromosomes distinctly, while other shows only *L* chromosome.

FIGS. 46–49.—Four nuclei in metaphase from same tetrad, in which two of them (48 and 49) show both *L* and *m* chromosomes, while other two show only *L* chromosome.

FIGS. 50, 51.—Two typical side views of anaphase, taken from same tetrad; one shows both *L* and *m* chromosomes, while other shows only *L* chromosome.

FIG. 52.—Tube nucleus and generative cell in telophase.

FIG. 53.—Tube nucleus proceeding to resting stage, generative cell about same stage, spindle resolving into cytoplasm, and thick wall of microspore.

FIG. 54.—Polar view of prophase stage from stem tip of male sporophyte, in which two *L* and *m* chromosomes well indicated.

FIG. 55.—Side view of anaphase stage from very young leaf in branch tip of female sporophyte; two *L* chromosomes noticeable.

FIG. 56.—Typical chromosome group in prophase, taken from branch tip of female sporophyte, in which two *L* chromosomes are shown.

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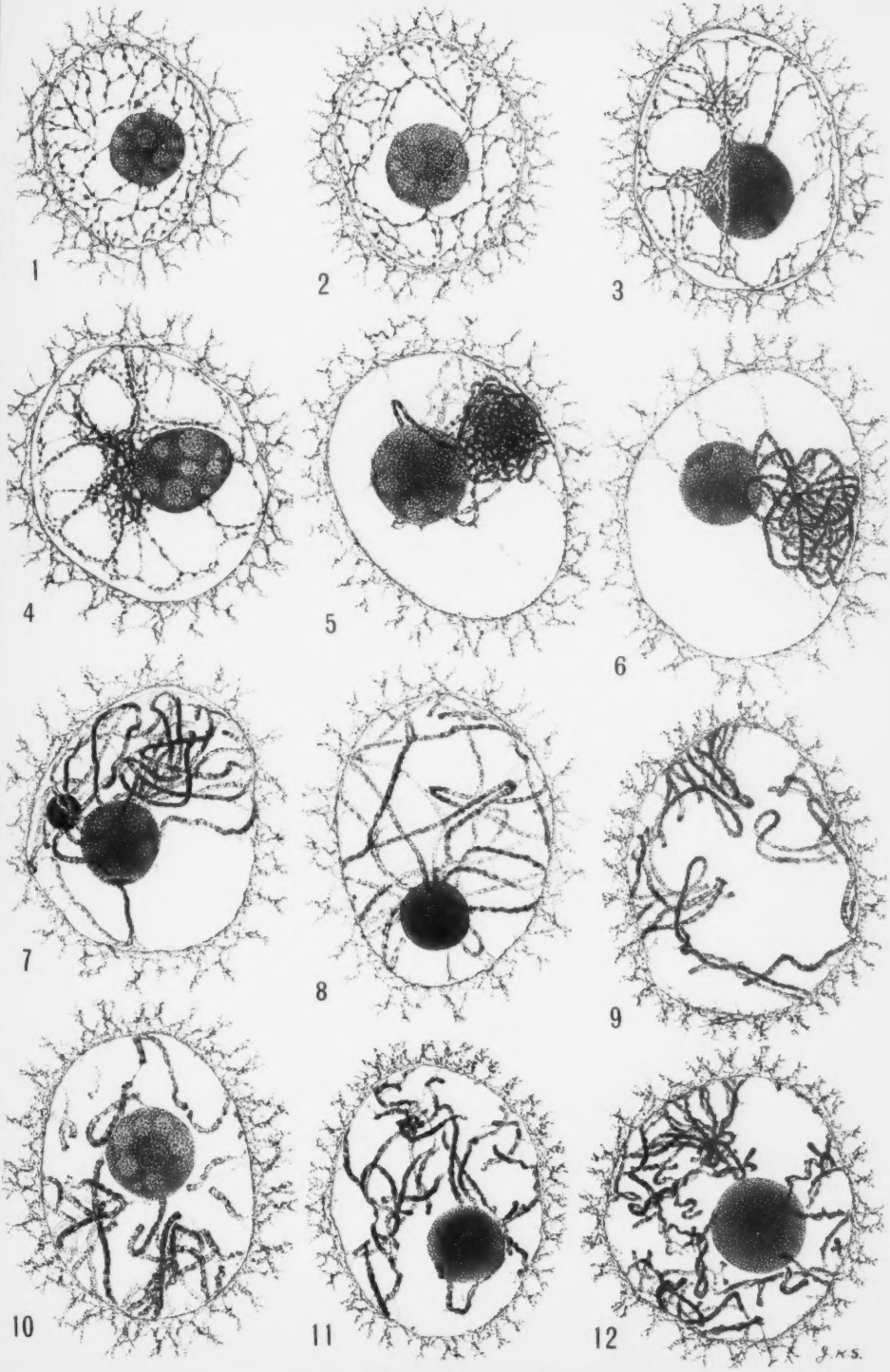

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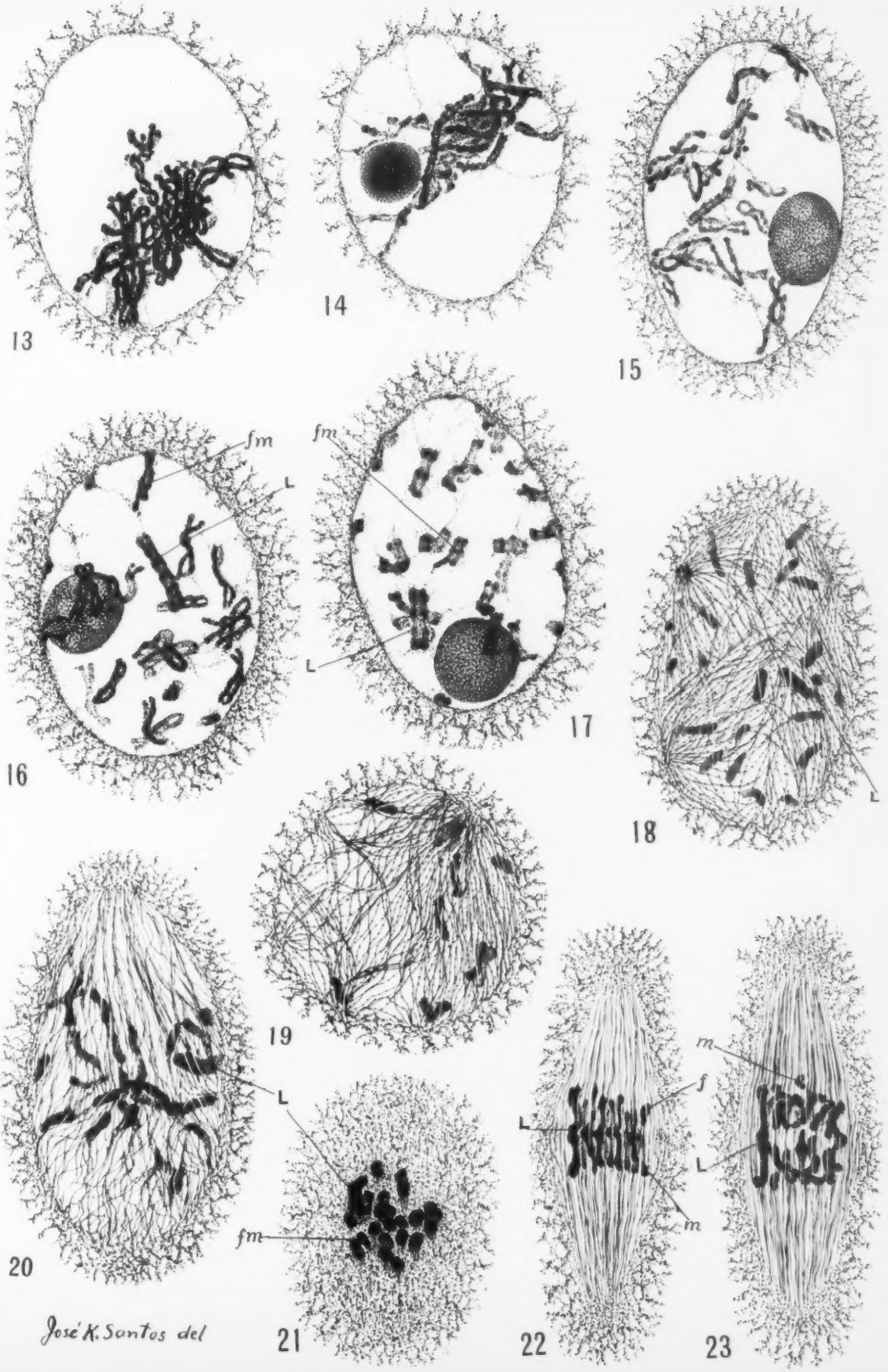

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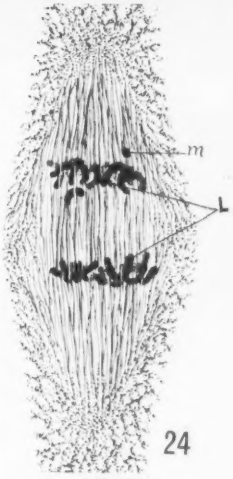

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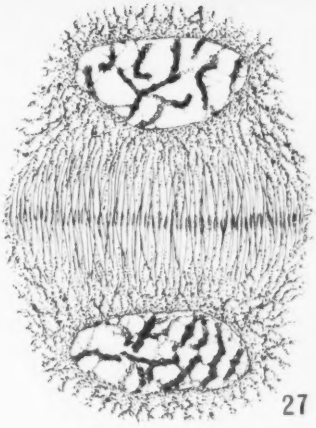

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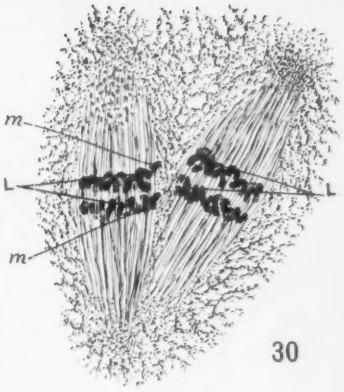

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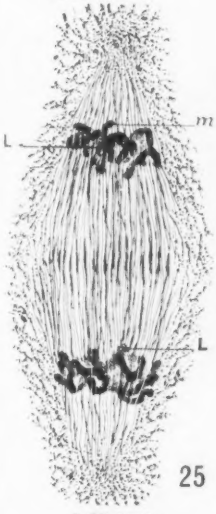

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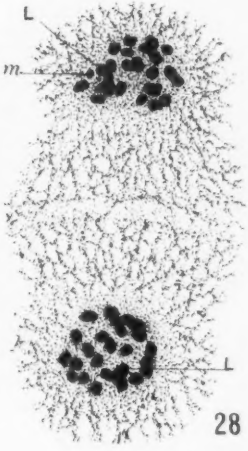

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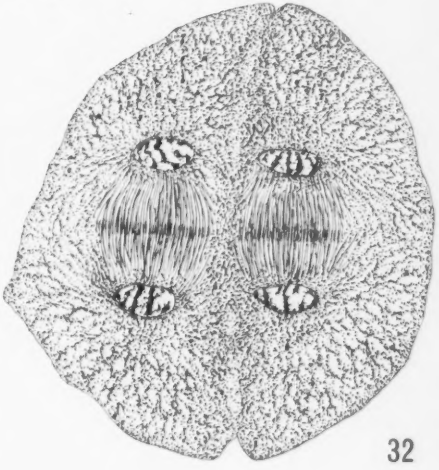

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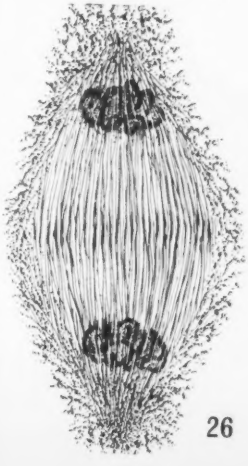

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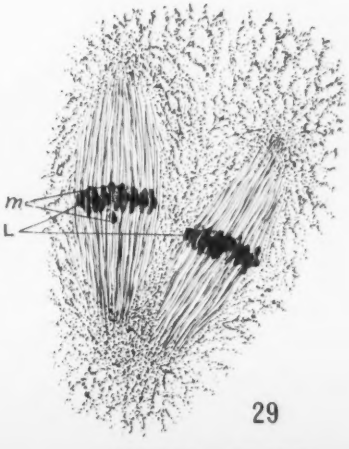

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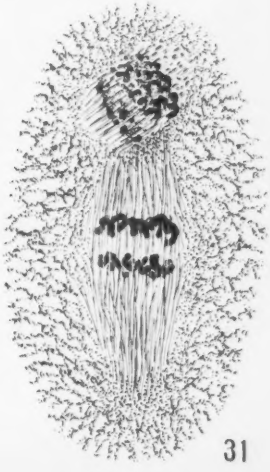

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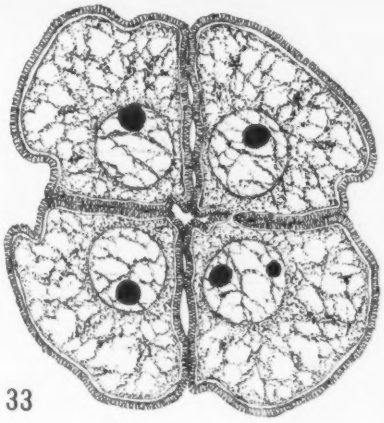

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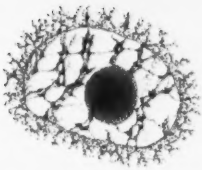

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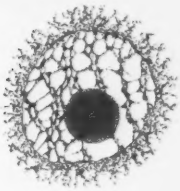

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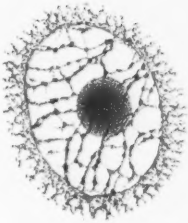

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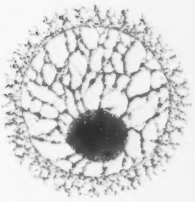

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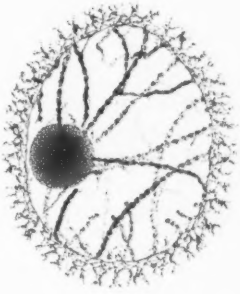

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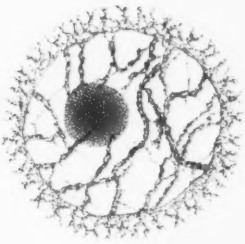

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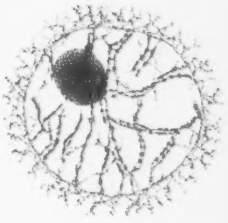

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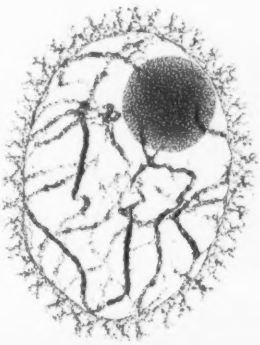

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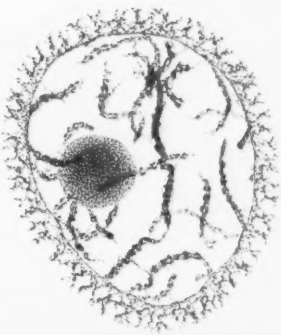

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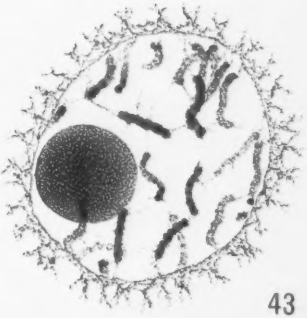

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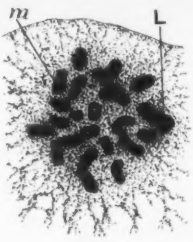

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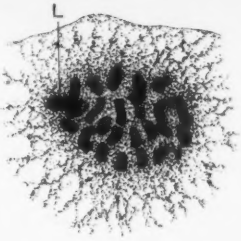

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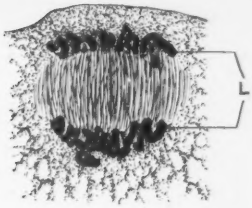

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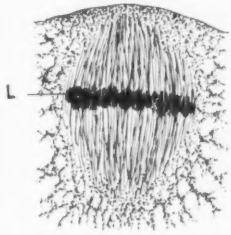

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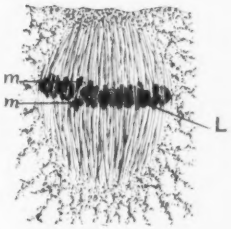

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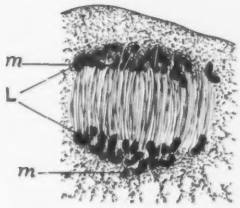

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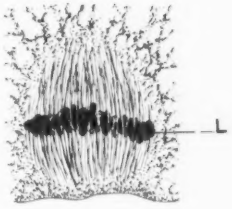

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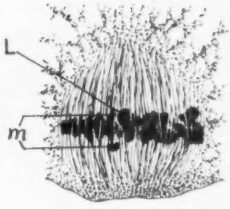

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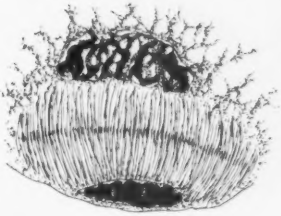

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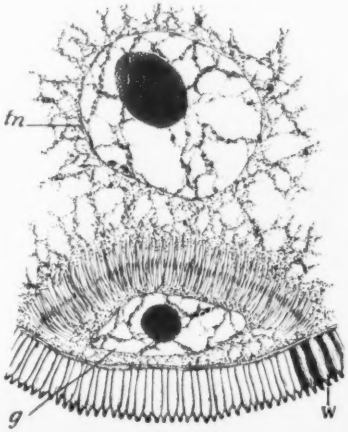

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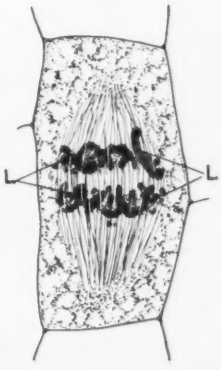

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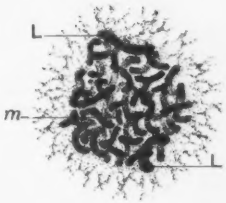

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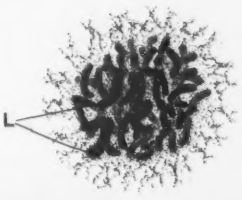

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José K. Santos del.

SANTOS on ELODEA

PROTEIN SYNTHESIS BY PLANTS

I. NITRATE REDUCTION[1]

SOPHIA H. ECKERSON

Introduction

Many workers have studied protein synthesis and some progress has been made, but there are still so many gaps in our knowledge that it is impossible to form a clear idea of the process. There is considerable confusion and some contradictory evidence in the literature of the subject. For instance, the effect of light has been a mooted question from the beginning. One says "Light is necessary"; one "Light is not necessary"; and the next "Is light necessary?" It may be that light is necessary for one of the steps but not for others; or it is possible that light, even though not necessary, may greatly influence the rate at some part of the process. It is hoped that by following the process step by step on the same kind of plants and with the same technique, it may be possible to clear up a few of the much discussed points and thereby add a little to our knowledge of the process as a whole.

In this study of protein synthesis there are three distinct phases which will be considered: (1) reduction of nitrates; (2) synthesis of amino acids; and (3) linking together of amino acids. This paper deals with the first phase.

Material and methods

In a study of protein synthesis one must guard against the possibility of mistaking regenerated proteins for those newly synthesized, and amino acids from the hydrolysis of proteins for newly synthesized amino acids. Such a possibility of confusion is reduced to a minimum by the use of carbohydrate-high tomato plants, grown as described by KRAUS and KRAYBILL (15), as experimental material. They state as follows:

> Plants grown with an abundant supply of nitrogen and then transferred and grown with a very low supply of available nitrogen are very weakly vegeta-

[1] The work is being carried on at the University of Wisconsin, in cooperation with Professor E. J. KRAUS.

tive and unfruitful. As compared with the vegetative plants, they are very much lower in moisture and total nitrogen and are lacking in nitrate nitrogen; they are much higher in total dry matter, free-reducing substances, sucrose, and polysaccharides. On supplying nitrate to the soil, such plants . . . . first began active growth at the stem tips. This was associated with a greening of the younger leaves and a very rapid disappearance of the starch grains from the pith cells of the stem, first near the tip and then progressively down the stem to its very base.

It seems that such plants must have all the essential substances and conditions necessary for protein synthesis, except available nitrogen, since new growth begins immediately upon the addition of nitrates. Any amino acids appearing in these low-protein plants soon after the addition of nitrates are almost certainly newly synthesized. This is to be differentiated from the increase in soluble nitrogen in carbohydrate-high plants in the dark (NIGHTINGALE), and from the production of new shoots at the base of carbohydrate-high plants after the tops have been removed (**15**). Another advantage is that, since there is an abundance of carbohydrates, the plants can be kept in darkness for several days without danger of sugar depletion.

Tomato plants of the Bonny Best variety were grown in rich soil until about 8 inches high, then transferred to quartz sand and grown until typically carbohydrate-high in appearance. The stems were stiff, the younger leaves small, somewhat yellow, and growth at the tip had stopped. The older leaves frequently were purplish in color, due to the presence of anthocyanin. The plants were watered daily with city water, and in addition twice a week with dilute nutrient solution lacking nitrogen.

A microchemical examination of these carbohydrate-high plants was made. Calcium nitrate was then supplied to the sand in five pots (ten plants) and the subsequent chemical changes followed closely by microscopic and microchemical examinations. Frequent examinations were made of both nitrogen-high (carbohydrate-low) and carbohydrate-high (nitrogen-low) plants for comparison. Two weeks later another lot of ten plants was given calcium nitrate. This was continued until there were several series, each at a different stage of vegetative growth.

Microchemical tests

NITRATES.—1. Blue color with diphenylamine in sulphuric acid (0.1 gm. diphenylamine dissolved in 10 cc. sulphuric acid, 3 parts conc. H_2SO_4 to 1 part water). 2. Crystallization as potassium nitrate in sections of the tissue in absolute alcohol on the slide. Identification by polarized light.

NITRITES.—1. Red color with sulphanilic reagent (*a*, sulphanilic acid 0.5 gm., acetic acid (33%) 150 cc.; *b*, alphanaphthylamine 0.1 gm., distilled water 20 cc., acetic acid (33%) 150 cc.). Dissolve the alphanaphthylamine by warming in 20 cc. water. Combine solutions *a* and *b* and keep in a tightly stoppered bottle (FRED 13). 2. Crystallization as silver nitrite.

AMMONIA.—1. Yellow color with Nessler's reagent. 2. Crystallization as ammonium magnesium phosphate.

P_H VALUE.—The Clark and Lubs indicators made up according to directions given by CLARK (10) for aqueous solutions of the alkali salts were used.

RESULTS

BEFORE ADDITION OF NITRATES.—When the carbohydrate-high plants were ready for experimentation they contained an abundance of glucose, some fructose, and a little sucrose. In the stem pith and endodermal and cortical cells were filled with starch. In the leaves the parenchyma cells, even of the very youngest leaves, were packed with starch grains. The tissue gave no reaction for nitrates, nitrites, or ammonia. No amino acids could be detected.

AFTER ADDITION OF NITRATES.—Within twenty-four hours nitrates were present in all parts of the plants. The tops of a few plants gave a slight reaction for nitrites. There was no reaction for ammonia.

Within thirty-six hours all the plants had considerable nitrite, strictly localized in the following regions: *a*, in the cortical cells at the tips of the stems; *b*, in the cortical cells of the petioles and near the veins of the younger leaves; *c*, in the stem (at the nodes), in the cortical cells near the phloem, and in the phloem parenchyma. In all these regions there was a trace of ammonia. Within forty-eight hours there was slightly less nitrite but more ammonia.

A few crystals of asparagin appeared on putting sections in absolute alcohol. At this time there was a slight but definite decrease in the amount of starch in the cortical cells at the tips of the plants and from the youngest leaves. Within three to five days there was very much less nitrite, a little ammonia, but a great increase in amino acids. Succinic acid and malic acid were now present, as well as the following amino acids: aspartic acid, asparagin, alanine, leucine, cystine, histidine, and at least two not yet identified. Simultaneously with this appearance of amino acids starch was disappearing and the chloroplasts of the younger leaves were becoming greener. Externally the plants showed signs of new growth at the tips. These and further developments are described fully by KRAUS and KRAYBILL (15).

Within three days following such transfer (from no nitrogen to available nitrogen) the beginning of the disappearance of the starch grains from the center pith cells and cortical cells at the tips of the plants was very noticeable. Successive examinations as growth progressed showed an active terminal elongation which contained no storage starch except in the starch sheath, an active development of secondary xylem in the older portion of the stem, and a very rapid, progressive, and finally complete disappearance of the starch from the pith and xylem parenchyma and also the cortical cells even down to the bases of the stems, where it was the last to disappear.

Beyond the first five to ten days after nitrates had been supplied there was only a little nitrite at any time. There were even occasional times when none could be detected in a few of the plants. Ammonia was always present in small amounts, from a trace to a little more. Amino acids apparently increased in amount up to about the third week.

P_H VALUES.—The P_H values for the different tissues of tomato plants which had just been supplied with nitrates proved to be very interesting. In a cross-section of the stem the values were approximately: center pith cells 5.2–5.0, xylem cells 4.6–4.4, phloem parenchyma and inner cortical cells 7.2–7.6. In the younger leaves the values were approximately: xylem and adjacent parenchyma cells and cortical cells 5.8–5.6, phloem parenchyma and nearby cortical cells 7.2 to 7.4 or 7.6; that is, the cells where starch was being hydrolized were slightly acid, while the cells where nitrate was being reduced were slightly alkaline.

Reduction of nitrates by plant extract

PLANT POWDER.—The leaves of nitrogen-high tomato plants were removed and kept separate from the stems, which were separated into nodes and internodes. These were cut into small pieces and ground fine in a meat grinder. Each lot was squeezed through folded cheesecloth. The juice was then treated with 30 per cent alcohol, filtered, and the filtrate precipitated with 95 per cent alcohol. The precipitate was taken up in water, again precipitated with 95 per cent alcohol, then once more taken up in water and precipitated with 95 per cent alcohol. This final precipitate was dried

TABLE I

REDUCTION OF NITRATES IN ALKALINE SOLUTION, ACCELERATED BY ADDITION OF PLANT POWDER; 10 CC. 2 PER CENT KNO_3 PLUS CARBOHYDRATE WITH OR WITHOUT $NaHCO_3$ WITH OR WITHOUT 0.1 GM. LEAF POWDER PLUS TOLUOL; 20 HOURS IN DARKNESS AT 50° C.

Carbohydrate	With or without Na_2CO_3	P_H value	With or without plant powder	Nitrite (mg. per 100 cc.)
1 cc. 10 per cent				
1. acetaldehyde	Plus	7.2	Leaf	2.0
2. acetaldehyde	Minus	5.2	Leaf	Trace(?)
3. acetaldehyde	Plus	7.2	Leaf (boiled 2 minutes)	2.5
4. acetaldehyde	Plus	7.2	Internodes	2.5
5. acetaldehyde	Plus	7.2	Nodes	1.6
6. acetaldehyde	Plus	7.2	Minus	0.7
7. fructose	Plus	7.2	Leaf	1.6
8. glucose	Plus	7.2	Leaf	1.0
9. acetaldehyde	Plus	7.2	1 gm. leaf	5.0

on the filter paper, then the dry snow-white powder was scraped off and kept in stoppered vials, in darkness.

It was found that small amounts of this plant powder accelerated the rate of reduction when added to an alkaline solution containing nitrates and some easily oxidizable carbohydrate. There was very little or no reduction in acid solution. The results given in table I are typical. In the series the amount of plant powder used in each case was 0.1 gm. for 10 cc. potassium nitrate. If this amount was increased the rate of reduction increased, but not in proportion, as shown in 9 at the foot of the table.

Many series of experiments were made to determine the most favorable hydrogen-ion concentration. One of the later series is

given in table II. It will be more profitable to discuss these results after the presentation of data concerning experiments with plant juice.

PLANT JUICE.—The juice of nitrogen-high and of carbohydrate-high tomato plants was extracted by grinding up the tissues and squeezing through folded cheesecloth. Toluol was added at once to prevent bacterial action.

This fresh juice had an exceedingly high reducing activity. The juice from nitrogen-high plants contained an abundance of both nitrates and sugars. It was only necessary, therefore, to reduce the hydrogen-ion concentration (P_H 6.4) to approximately P_H 7.6 and reduction began at once. The carbohydrate-high plants

TABLE II

EFFECT OF HYDROGEN-ION CONCENTRATION ON REDUCTION OF NITRATES; 10 CC. 2 PER CENT KNO_3, CARBOHYDRATE, N/10 NaOH, AND 0.1 GM. LEAF POWDER WITH TOLUOL; IN DARKNESS AT 50° C.

Carbohydrate	N/10 NaOH (cc.)	P_H value	Nitrite (mg. per 100 cc.) 24 hours	Nitrite (mg. per 100 cc.) 36 hours
1 cc. 10 per cent				
1. acetaldehyde	0.6	7.0*	1.0	1.5
2. acetaldehyde	0.75	8.4*	3.3	7.5
3. acetaldehyde	0.8	9.2	1.8	1.0
4. 0.1 gm. fructose	0.2	8.4*	6.5	10.0
5. 0.1 gm. fructose	0.3	9.2	2.5	2.0

* At the end of 36 hours the P_H value of 1 was 6.8; of 2, 7.6; and of 3, 7.4.

contained an abundance of sugar but no nitrate. The juice of such plants gave no reaction for nitrites until after nitrate had been added and the whole made slightly alkaline. At a P_H value of 7.6 the reducing activity was even greater than that of the juice from nitrogen-high plants. Table III shows the rate of reduction at 50° C.

Boiling the juice considerably increased the acidity. Such acid juice reduced nitrates to nitrites only to a very slight extent, or not at all. In every case when the hydrogen-ion concentration was decreased to a P_H value of approximately 7.6, however, the reducing activity was as great as before heating. The longer the juice was boiled the more acid it became, and, naturally, the more

sodium was required to bring it to the P_H value 7.6. This is shown in table IV.

HYDROGEN-ION CONCENTRATION.—The most favorable P_H value for reduction of nitrates by plant juice was approximately 7.6. This value was maintained nearly constant (7.6–7.4) throughout

TABLE III

REDUCTION OF NITRATES BY JUICE OF NITROGEN-HIGH (N+) AND CARBO-HYDRATE-HIGH (C+) TOMATO PLANTS; IN DARKNESS AT 50° C.; TOLUOL ADDED IN ALL CASES

PLANT JUICE	N/10 NaOH PER 10 CC.	P_H VALUE	NITRITE (MG. PER 100 CC.)		AMMONIA 20 HOURS
			3 hours	20 hours	
1. 20 cc. N+	0.5	7.6	2.5	50.0	Trace
2. 20 cc. N+	0.0	6.4	1.0	5.0	None
3. 10 cc. C+	0.2	7.6	0.0	Trace?	None
4. 10 cc. C+ and 10 cc. 2 per cent KNO_3	0.3	7.6	3.3	40.0	Trace
5. 10 cc. C+ and 10 cc. 4 per cent KNO_3	0.4	7.6	3.3	60.0	Trace
6. 10 cc. C+ boiled 10 minutes and 10 cc. 4 per cent KNO_3	0.0	3.0	0.0	00.00	None
7. 10 cc. C+ boiled 10 minutes and 10 cc. 4 per cent KNO_3	1.2	7.6	5.0	50.0	Some

TABLE IV

INCREASE OF ACIDITY OF TOMATO PLANT JUICE ON HEATING; 10 CC. CARBOHYDRATE-HIGH (C+) JUICE, BOILED, AND N/10 NaOH TO BRING TO P_H 7.6

C+ juice boiled (minutes)	N/10 NaOH per 10 cc.	P_H value
1. 0.0	0.20	7.6
2. 0.5	0.30	7.6
3. 2.0	0.75	7.6
4. 5.0	0.90	7.6
5. 10.0	1.20	7.6
6. 15.0	1.50	7.6

the experiment, probably owing to the presence in the juice of inorganic salts which acted as buffers. On the other hand, solutions of the plant powder containing little inorganic salt quickly became more acid on oxidation of the aldehyde or sugars. When the P_H value at the start was 7.6, in a short time it was 7.2 or 7.0 when

reduction was greatly decreased. The greatest reduction, however, was obtained when the value at the start was 8.4 and at the end of 20 hours 7.6–7.4. It seems, therefore, that the most favorable P_H value for reduction of nitrates by plant powder as well as by plant juice must be near 7.6. It will be remembered that the P_H value of the cells of the living plant when reduction of nitrates was taking place was usually 7.4 to 7.6, although occasionally 7.2 or 7.8.

LIGHT.—In all of the earlier experiments check series were run in both light and darkness. I was never able to detect any influence of light on either the rate or the amount of reduction of nitrates by plant extract (powder or juice). In the living plant there is reduction of nitrates in darkness, and for the first two or three days, seemingly at about the same rate as in light. After that differences appear, the exact nature of which is not yet clear. This is being studied further in connection with amino acid synthesis.

OXYGEN.—It was early found that any shutting out of air from the surface of the reacting solutions greatly decreased the amount of reduction, indicating that some free oxygen is required. In all of the experiments described in this paper the reacting solutions were in 50 cc. flasks, thus allowing free access of air to the relatively large surface.

PLANT POWDER.—Since the activity of the plant juice far exceeded that of the plant powder, much must have been lost somewhere in the process of preparing the powder. It was found to be in the final precipitation with 95 per cent alcohol. An attempt was made to prepare a more active powder by eliminating this step. Carbohydrate-high tomato plant juice was squeezed through four-fold cheesecloth, precipitated with 30 per cent alcohol, and filtered. After evaporation of the alcohol this filtrate was highly active. It was evaporated to dryness in 10 cc. lots at 50° C., and the reducing activity tested. It is not known how long this dry material would retain its activity. There was no loss at the end of five days, when the last lot was tested. A single experiment will suffice to show the activity: C+ juice after precipitation with 30 per cent alcohol. Ten cc. filtrate evaporated to dryness at 50° C., taken up in 10 cc. water+0.2 gm. KNO_3+0.1 gm. fructose+0.6 cc. N/10

NaOH (to give P_H 7.6) in 20 hours at 50° C. gave 50.0 mg. nitrite per 100 cc.

Discussion

REDUCASES.—In 1886 REY-PAILHADE discovered an enzyme in yeast that reduces sulphur by adding hydrogen. This he named philothion. Later, POZZI-ESCOT (**19**) found that philothion also reduces nitrates to nitrites. In 1902 SCHARDINGER (**20**) found an enzyme in fresh milk which reduces methylene blue to its leucobase. Later, BACH (**2**) found that the Schardinger enzyme also greatly accelerated the reduction of nitrates to nitrites by an aldehyde. BACH (**3**) also found that an enzyme could be extracted from calf liver which accelerates the reduction of nitrates in the presence of acetaldehyde. So far as I have been able to find, there are only three workers who have made a study of the reduction of nitrates by reducases obtained from plants: POZZI-ESCOT (**19**) from burdock stems; KASTLE and ELVOVE (**14**) from potato tubers and etiolated sprouts, and egg plant fruit; and BACH (**5**) from potato tubers. BALY, HEILBRON, and HUDSON (**6**), and BALY, HEILBRON, and STERN (**7**) have studied the reduction of nitrates and the simultaneous oxidation of formaldehyde in ultra violet light. Although these BALY papers are valuable, they should be read with caution, as they contain much that is purely hypothetical. Finally, there is a series of papers by BAUDISCH (**8, 9**) on the reduction of nitrates in the presence of glucose and traces of iron.

REACTION OF MEDIUM.—The reaction of plant extracts depends largely upon the plant, but also upon the method of extraction and subsequent treatment. The P_H value of nitrogen-high tomato plant juice immediately after being squeezed through cheesecloth was 6.4, after filtration through asbestos 6.0, after precipitation with 30 per cent alcohol 5.8. To bring all to the same P_H value different amounts of hydroxide or of carbonate must be added. In previous work no exact determination of the acidity or alkalinity of the plant extracts was made. For this reason it is difficult to interpret some of the data. POZZI-ESCOT (**19**) found that the solution of the yeast enzyme (philothion) had a slight acid reaction. The addition of traces of alkali gave a very active solution. The "addition of any considerable amount of a mineral acid or a strong organic acid

(acetic) acts as a strong paralyzer." Of the salts, "the most energetic paralyzers are those with an acid reaction." This indicates that this reducase is most active in a slightly alkaline solution, which agrees with the present work with tomato reducase. KASTLE and ELVOVE (**14**), working with unfiltered potato extract, state that probably a slightly acid medium is best, as when sodium hydroxide or lime water are added "to neutralize" there is no reduction. They used 1 cc. N/10 NaOH to 10 cc. potato extract, and 2 cc. of 50 per cent KNO_3. Unless the potato extract was more acid than tomato plant juice, this would seem to be too much alkali. BACH (**3**) states that the addition of sodium bicarbonate (1 gm. to 100 cc.) had no effect on the reduction of nitrates in the system, that is, reducase (from calf liver or milk) plus $NaNO_3$ plus acetaldehyde. BACH apparently was working with neutral solutions, as twice in the process of extracting reducase from calf liver he treated with sodium bicarbonate, then neutralized with acetic acid.

Without exception, every one who has studied the reducing enzymes states that heating (90–100° C.) for 3–5 minutes destroys the activity. Heating plant juice increases the acidity. Tomato plant extract was as active in reducing nitrates after boiling as before, provided the solution was made slightly alkaline.

POZZI-ESCOT compared catalase with philothion, and found them similar in several characteristics. Both have the ability to split off oxygen from hydrogen peroxide and both reduce nitrates. He concludes that catalase is a reducase. It has been shown (**1, 11, 16**) that catalase is more active, in splitting off oxygen from hydrogen peroxide, in an alkaline solution.

Oxidations and reductions go on at the same time. If one substance is reduced another must be oxidized. DAKIN (**12**) considers that "reductions and oxidations are two expressions of one process, dehydration." He prefers the term dehydrase to reducase. For the process of reduction, and accompanying oxidation, some easily oxidizable substance is necessary, also free oxygen. The early workers added an aldehyde to solutions containing nitrate and animal or plant extract. BACH (**3**) found that acetaldehyde was better than formaldehyde. BALY, HEILBRON, and HUDSON (**6**), in their work on the reduction of nitrates in ultra violet light, used

formaldehyde. They think that nitrates are reduced in the light by activated formaldehyde in green leaves. In their opinion newly synthesized formaldehyde is in a highly active state.

This theory could scarcely be considered in the case of carbohydrate-high tomato plants. Here it is the excess stored carbohydrates that is used up rapidly when nitrates are present. Fructose and glucose are oxidized, some perhaps in increased respiration, but much of them to form amino acids. As rapidly as fructose and glucose are used, more starch is hydrolyzed. In the experiments with tomato plant powder, therefore, the effectiveness of fructose and glucose was tested in comparison with acetaldehyde. In the early experiments, where the reaction was about neutral, the order according to the amount of nitrite produced was acetaldehyde > fructose > glucose, but at a P_H value of 8.4–7.6 the order was fructose > glucose > acetaldehyde. BAUDISCH (9) found glucose effective in the reduction of nitrites. He states that "the smallest trace of an iron salt is sufficient to reduce a large amount of nitrite on warming with glucose in weakly alkaline solution." That oxygen also is necessary has been shown by several workers. DAKIN states that methylene blue could not be decolorized by the dehydrase (reducase) of milk in an atmosphere of hydrogen. BAUDISCH "found that ferrous bicarbonate, or hydroxide, in the absence of oxygen even at the temperature of boiling water does not attack alkali nitrates to the slightest extent; the presence of oxygen, however, brings about immediate reduction to nitrite, and there is a direct relation between the amount of oxygen dissolved in the water and the amount of nitrite formed." There was no reduction of nitrates by tomato plant extract if the solution was in small vessels filled, for the exclusion of air, and stoppered.

BALY and associates find that in their experiments reduction of nitrates takes place only in ultra violet light, but they think light is necessary only to activate the formaldehyde, that the reduction itself is not a photochemical process. BAUDISCH (9) found that "in the case of cholera bacteria the reduction proceeds just as rapidly in the dark as it does in the light." Here he found the reduction of nitrates to nitrites is in direct relation to the oxygen respiration of the bacteria and to their iron content. The rate of

reduction of nitrates by tomato plant extract in darkness was the same as in light. There was reduction of nitrates by tomato plants in darkness, and for the first two or three days, at least, it seemed to go on at about the same rate as in the light.

From the foregoing results there seems but little question that there is in tomato plants an active substance which, on being added to an alkaline solution of potassium nitrate containing fructose or glucose, causes rapid reduction of nitrates to nitrites, and a slower reduction of nitrites to ammonia. The nature of this substance is not known. Is it an enzyme of organic nature, or is it an inorganic substance? It is not impossible that it may be iron, and the chemical process may be that suggested by BAUDISCH (**9**). If it were iron, however, judging from the work of NEUBERG (**17, 18**) on the catalytic action of sunlight in the presence of small amounts of iron compounds and from my own work with light-sensitive seeds, I should expect an increased activity in light. It may be possible in the future to determine the nature of the active substance.

Summary

1. Reduction of nitrates to nitrites and ammonia was obtained by tomato plant extract in slightly alkaline solution in the presence of fructose or glucose and some free oxygen in darkness as well as in light.

2. The reduction was most active in solutions having a P_H value of approximately 7.6.

3. The expressed juice of nitrogen-high tomato plants (containing both nitrates and sugar in abundance), after adding N/10 NaOH to bring to a P_H value 7.6, gave a very rapid reduction of nitrates to nitrites at 50° C.

4. The expressed juice of carbohydrate-high tomato plants (containing much sugar but no nitrates), brought to P_H 7.6, gave no reaction for nitrites after 20 hours at 50° C. Upon addition of nitrates there was even more rapid reduction than by the nitrogen-high juice.

5. Tomato plant juice boiled and brought to a P_H value 7.6 reduced nitrates as rapidly as the unheated juice.

6. The rate and amount of reduction of nitrates to nitrites by tomato plant extract were exactly the same in darkness as in light.

7. Probably the rate of reduction of nitrates to nitrites by carbohydrate-high tomato plants in darkness was the same as in light for the first few days. Then disturbing differences appeared which are not yet understood.

8. In carbohydrate-high tomato plants the reduction of nitrates was localized in the following regions: at the stem tip just behind the growing region; in the leaf cells, especially near the phloem and in the cortical cells of the petioles; in the stem, especially near the nodes, in the phloem parenchyma, and in cells in the cortex near the phloem.

9. All these regions were slightly alkaline in reaction. The pith cells were acid. In the carbohydrate-high plants before nitrate was supplied there was less difference. The pith cells were slightly acid while the phloem parenchyma was about neutral in reaction.

10. Amino acids (newly synthesized) appeared at the nodes and in petioles and blades of young leaves and just behind the stem tips of carbohydrate-high tomato plants three or four days after nitrate had been supplied to the soil. The amino acids were aspartic acid, asparagin, alanine, leucine, cystine, and histidine.

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FEMALE GAMETOPHYTE OF MICROCYCAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 318

LILLIAN GRACE REYNOLDS

(WITH PLATES XXVIII, XXIX AND SIX FIGURES)

Microcycas calocoma is an endemic genus with a single species, occurring in mountainous regions of Pinar del Rio in western Cuba. It was first described by MIQUEL (14), whose description was amplified by DE CANDOLLE (9). In 1907 CALDWELL and BAKER (2) published the first adequate account, and a few months later CALDWELL (1) gave the first satisfactory description of the genus, and the first account of its life history. The name *Microcycas* proved to be a misnomer, for it is a tree, only two other genera of cycads having taller species.

The material for this paper was collected by Professors CALDWELL and CHAMBERLAIN during the years 1907–1911, but at different seasons. Professor CHAMBERLAIN collected still other material in 1914, and again in 1922. The sections from which the study was made were prepared by CALDWELL, Miss PACE, YAMANOUCHI, and myself. The earlier sections were stained in gentian violet, the others in Haidenhain's iron-alum haematoxylin, and to those most recently made, gold orange was added to bring out the cell walls. I am under great obligation to Professor CHAMBERLAIN for suggestions and criticisms during the progress of this investigation, and to P. J. SEDGWICK for valuable assistance in the making of the photomicrographs.

Development of endosperm

YOUNG GAMETOPHYTE.—The youngest stage found in the development of the female gametophyte of *Microcycas* shows free nuclear division in the megaspore (fig. 7). The material, although fixed in August, is not young enough to show the megaspore mother cell or the megaspores. The ovules are about 2 mm. long (fig. 8), and the gametophyte within is about half that length. The gameto-

phytes of this size vary somewhat in the number of free nuclei, the lowest number noted being 64, the next 256, all in early anaphase, but not suitable for counting the individual chromosomes; while in the gametophyte in the other ovule on this same sporophyll there has been one more mitosis, so that the number theoretically is 512. In another ovule of the same date, there has been one more division, so that the theoretical number of nuclei is 1024, but at this stage some of the nuclei are almost sure not to divide, so that the theoretical number is seldom or never reached. Considering the size of the gametophyte, there are probably no more nuclear divisions before the walls come in.

The early stages in wall formation, showing the first walls of the gametophyte striking in toward the center, might have been found in September ovules had they been available. The gametophyte in early October is about 3 mm. long, and shows that the peripheral nuclei are completely inclosed within walls. The gametophytes examined are badly shrunken, probably on account of the killing agent, and it is difficult to determine whether the nuclei in the center are free, or whether their walls are so delicate that they cannot be identified. In material dating from the middle of October to November, the female gametophyte is completely cellular (fig. 9).

MATURE GAMETOPHYTE.—From about November 22 to early December, the archegonial initials appear. In CHAMBERLAIN'S most recently collected material, the archegonial initials appeared in September, which is two months earlier than in ovules previously examined. This may be due to variance in the seasons of different years. The length of the ovule by this time is approximately 1 cm., and the length of the gametophyte 4–5 mm. Transverse sections show the cells arranged in regular rows converging toward the center (fig. 11). The peripheral cells are smaller than the interior cells, and are practically isodiametric. These peripheral cells are the result of many transverse and vertical divisions of the original cell. Those that are nearest the original walls apparently do not divide as frequently, and often have less cytoplasm than those in the middle. From these last are differentiated the archegonial initials (fig. 13).

Although not evident in ovules before this date, there now appears a "median cleft" (CALDWELL 1), or "closure" (Miss CAROTHERS 3), or suture. Cleft is an unfortunate term, since it implies the separation of parts once united, which is just the opposite of what is really the case. Closure is more apt, since it expresses the closing up of the central vacuole by the centripetal growth of the surrounding cells. Suture suits exactly the appearance of this median line as seen in stained sections. According to WEBSTER'S dictionary a suture is "the line or seam, formed by the union of two adjacent margins." Absolutely median sections of the mature gametophyte show the cells arranged in rows converging toward this suture (fig. 15). If the sections are not median, only the cells bordering on the original cell walls of the gametophyte appear in radiating rows. The others, being cut obliquely and transversely, appear as irregular patches of round cells bounded by cells in regular rows (fig. 16). Also, there are cracks or clefts connecting this suture with the exterior. These occur along the original walls, separating the progeny of one original cell from that of another. The extent of the progeny of each cell is visible on the surface of the gametophyte as a slight swelling, making the entire surface appear papillate.

The young cells of the gametophyte are uninucleate. In a mature gametophyte there may occasionally occur cells containing two nuclei, due to the breaking down of the walls between the two cells.

As a rule, the number of chromosomes is twelve, the number found in all the cycads which have been investigated; however, in two cases fourteen chromosomes were counted.

Nutrition of gametophyte

ENDOSPERM JACKET.—Around the youngest gametophyte examined there was a layer of cells about four or more cells thick (fig. 8). These cells are smaller than the cells of the integument, but similar to those of the nucellus in size of cell and size of nucleus. In other gametophytes of about the same age, this layer of four cells is differentiated into a layer two cells thick of plump cells immediately surrounding the gametophyte, and a layer outside of that of flattened cells fitting loosely together. There is a sharp

line of demarcation between these latter and the cells of the nucellus. The inside layer of cells is to become the nutritive jacket of the gametophyte. These cells are large, flat, and full of cytoplasm in which are imbedded large nuclei, nearly half the diameter of the cells. By October the walls are no longer thin, having thickened appreciably, while the cells themselves are still plump (fig. 10). The nutritive jacket cells around the gametophyte in November have very thick walls, on the inside of which are granules laid closely together (fig. 13). The nuclei are not so large in proportion to the size of the cell as in the preceding stages. In gametophytes a few days older, the cells of the jacket layer have become flattened, the walls are very thick, the granules are prominent, and the nuclei are much smaller. By the middle of December the nutritive jacket is practically all gone (fig. 17), only such vestiges as pieces of the granular cell walls remaining. At this time, too, the cells of the nucellus immediately above the archegonia are gone, and those above these are represented only by their cell walls.

CONTRIBUTION FROM SPOROPHYTE.—The vascular bundles belonging to the inner fleshy layer often branch just below its union with the nucellus, one strand continuing in the inner fleshy layer, and the other extending into the free portion of the nucellus, thus contributing directly to the nourishment of the gametophyte. Spiral elements in the bundle can be distinguished among the elongated, but as yet unmodified procambial cells as early as the archegonial initial stage. In the mature seed, when the inner fleshy layer of the integument and the nucellus have become papery, these branches can be seen like frayed ends extending 1.5–2 mm. into the almost transparent nucellus.

MEGASPORE MEMBRANE.—Meanwhile the wall of the megaspore becomes much modified, and is a noticeable membrane adhering closely to the gametophyte. The diameter of the megaspore membrane is smallest during the free nuclear division of the gametophyte (fig. 7), when it is approximately 0.5 μ wide; and 3 μ wide (its greatest width) about three months later when the gametophyte has just become cellular (fig. 9). From this time the archegonia are developing very rapidly, and the strain of the expanding tissue stretches the membrane at the micropylar end until it measures

only 2.5 μ during November (fig. 13), 1.5 μ during early December, and only 1 μ from the middle of December until the end of March, or just before fertilization. A measurement made of the membrane from the side of the gametophyte at this date is also 1 μ, as if the strain were not localized at the micropylar end, but felt all over. This is not inexplicable when the size of the mature gametophyte is considered.

In one of the ovules dated October 31, two gametophytes had developed (fig. 12). The centripetal growth of cell walls was not yet complete. Although both are inclosed by a common nutritive jacket, each is surrounded by an entire megaspore membrane, indicating individual origins from two megaspores, the only case of this kind yet reported for cycads.

Archegonia

DISTRIBUTION.—In the young gametophyte it is difficult to determine which cells should be regarded as archegonial initials, because all over the surface of the gametophyte are groups of cells not more than three or four cells apart, each cell containing a large nucleus and an abundance of cytoplasm (fig. 14). That these all may be potential archegonia is shown by the fact that in the mature gametophyte as many as four, five, or six archegonial groups may be seen scattered on the surface, in addition to the large group at the micropylar end. Of these groups, however, the only one to progress so far as to have the ventral canal nucleus division occur in its archegonia is that group which is beneath the micropyle. The archegonia of the remaining groups begin to disorganize by the time the central cell has enlarged to three or four times its original size.

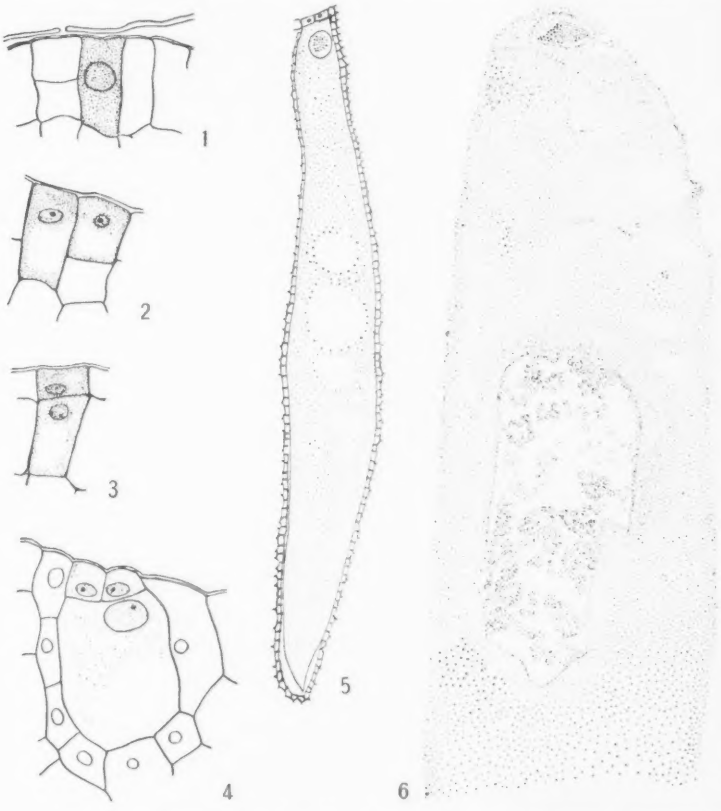
Not only are some archegonia scattered over the surface of the gametophyte, but occasionally they develop entirely inside, in groups or isolated. Generally they are at a considerable distance from the micropylar group, but do occur within this group itself, although at right angles to the other archegonia. In such archegonia as have been examined, the large central cell nucleus is imbedded in comparatively dense cytoplasm at one end, while the other end is very vacuolate, as in ordinary archegonia. The neck cells

were indistinguishable from the other cells of the gametophyte. That any of these archegonia definitely open on to sinuses seems improbable, but this could not positively be ascertained.

It would seem that the nourishment of the gametophyte might be one of the factors, or might be the particular factor, influencing the localization of the best developed archegonia at the micropylar end of the gametophyte. Not much food enters the gametophyte from the basal end of the ovule. The vascular bundles entering the inner fleshy layer branch very freely, and practically all of these branches terminate near the micropylar end of the gametophyte, so that this vascular system contributes more nourishment to this end of the gametophyte than to the basal. Then, also, the contents of the cells liberated by the formation and subsequent enlargement of the pollen chamber in the nucellus are probably absorbed, just as the cell contents are absorbed from the gametophyte jacket cells. Since this takes place at the micropylar end, there would be an increase in the food supply at this end of the gametophyte, thereby promoting the more rapid development of the archegonia here, and retarding and finally eliminating the archegonia scattered elsewhere.

DEVELOPMENT.—The first stage in the development of the archegonium is the differentiation of the archegonium initial from the other cells of the gametophyte. At first the initial is simply a cell better stocked with food and with a larger nucleus than the neighboring cells (fig. 1); then a vacuole appears below the nucleus and enlarges as the cell enlarges, the nucleus remaining at the top (fig. 2). The cytoplasm is likely now to look rather scanty in comparison with the size of the cell, because the increase in the mass of the cytoplasm does not keep pace with the increase in the size of the cell. The nucleus then divides to form the central cell and the primary neck cell (fig. 3), which probably divides almost immediately to form the two-celled neck (fig. 4). Within the next week the central cell enlarges immensely, becoming very vacuolate in the process (fig. 5). In early December, when the gametophyte is about 1 cm. in length, the archegonium is about twenty-four times the length of the original initial. A month later the vacuoles in the cytoplasm vary in size from very large ones in the basal end of the archegonium to very small ones near the neck, while in the

cytoplasm surrounding the nucleus there are none at all. As the archegonium matures, the density of its cytoplasm increases by



FIGS. 1–6.—Development of archegonium of *Microcycas calocoma:* fig. 1, archegonium initial; fig. 2, archegonium initial with vacuole in base before cutting off of neck cell; fig. 3, archegonium initial divided, giving rise to primary neck cell and central cell; fig. 4, primary neck cell divided to form two neck cells; fig. 5, central cell much elongated before division to form egg; fig. 6, ventral canal nucleus and egg nucleus; note that in this case a faint wall has been laid down on the spindle fibers and a portion of it has been dragged down into the cytoplasm of the egg; figs. 1–4, 6, ×238; fig. 5, ×52.

the concentration in the central cell of food received from the archegonial jacket and neighboring cells. At the time the ventral canal

nucleus is cut off, the cytoplasm appears to be very solid and without vacuoles.

VENTRAL CANAL NUCLEUS.—The division of the nucleus of the central cell to form the ventral canal nucleus and the egg nucleus takes place late in February (fig. 6). In one of the sections observed the division is complete, with the very much enlarged egg nucleus near the middle of the egg. Both nuclei have spindle fibers attached to them; and cutting off the ventral canal nucleus and its cytoplasm from the egg proper is a very faint wall developed on spindle fibers (fig. 6). A fragment of this wall with its fibers has been dragged down into the egg, and shows very clearly that what appeared to be one wall is really two walls. Ordinarily the ventral canal cell is not separated from the egg by a wall. This is the only cycad in which even a trace of a wall has been observed. As to whether it disorganizes or is permanent, there is at hand no material old enough to determine.

NUTRITION OF EGG.—By the time the ventral canal nucleus is formed, the egg is inclosed in a membrane perforated by haustoria which are protrusions of egg cytoplasm. These haustoria pass through the egg membrane in groups of two and three or more (fig. 18). The egg haustoria penetrate the cells of the archegonial jacket and through them the contents of these cells pass into the egg. Finally, these cells are practically exhausted, the cells being empty, scattered, flattened, and otherwise distorted.

The beginning of the archegonial jacket is barely recognizable when the primary neck cell divides to form two neck cells. Thereafter it develops in such a manner as to make it difficult, when looking at a micropylar group of archegonia, to determine whether it is individual for each archegonium, or a region in which the archegonia are imbedded. When, as it sometimes happens, however, one archegonium develops so as to project itself into another, there is always between the two central cells a single layer of cells, the archegonial jacket. Although present, the archegonial jacket in *Microcycas* is not as strongly developed, and does not last as long as in other cycads, being very much exhausted by the latter part of December.

ANOMALOUS ARCHEGONIA.—Numbers of the archegonial initials at the periphery of the micropylar group and scattered over the remaining surface of the gametophyte develop into abnormal archegonia. Some of these anomalous archegonia are multinucleate. This condition, as indicated by different stages found on sectioning, may arise in two ways: either by the division of the central cell nucleus before the greatest elongation of the archegonium and the subsequent cutting off of the ventral canal nucleus (figs. 21–23); or by fragmentation of the central cell nucleus at a period just before the separation of the ventral canal nucleus from the egg nucleus by cutting off of amoeboid arms (fig. 19).

Another variation in archegonia is in the number of neck cells. In one case it appears that the primary neck cell has divided transversely, and in another case, that each of the cells has divided vertically, forming two tiers of neck cells, two cells in each tier (fig. 21).

Generally the archegonia on the sides of the gametophyte are grouped without a definite archegonial jacket. These archegonial complexes probably originate from a group of adjacent archegonial initials, all of which develop, permitting no jacket to be formed for any one of them, but only around the group as a whole. In such groups there occur archegonia in which, after the division of the primary neck cell and the central cell, the latter divides several times like any other gametophyte cell. These archegonia are never as advanced as those at the micropylar end. Around some archegonia the jacket cells at the base have enlarged, resembling archegonial initials similar to the "sheath buds" described by Miss FERGUSON (11) in *Pinus*.

ARCHEGONIAL CHAMBER.—In all the material sectioned, except possibly in one group of slides, it seems that no archegonial chamber is formed. In examining some whole ovules, however, fixed about March, it would seem that the gametophytes previously sectioned had not been old enough, and that there is an archegonial chamber formed in the micropylar end by the continued growth of the gametophyte cells around the archegonia, but it is not a deep and definite chamber as is found in other cycads. The indication in the lateral groups of archegonia of an archegonial chamber is very slight.

Discussion

CALDWELL (1) considered *Microcycas* the most primitive genus of cycads. On the other hand, LAND (12) and CHAMBERLAIN (7, 8) conclude that it is an advanced form. In habit of growth it is a tree (1), like all but *Zamia*, *Stangeria*, *Bowenia*, some species of *Macrozamia*, and *Encephalartos*. The three first named are generally accepted as the most advanced cycads; therefore, in vegetative form *Microcycas* belongs to the lower cycads. In the seedling, it is to be noticed that the vascular cylinder, instead of developing from a protostelic cotyledonary plate, is siphonostelic from the beginning (10), an advanced character.

There is a tendency among cycads to reduce the microspore production by reducing the size of the sporophyll, by decreasing the number of sporangia on the sporophyll, and by diminishing the output of each sporangium. In all these respects *Microcycas* (15) is near the top of the series, with only *Bowenia* and possibly *Zamia* more advanced.

As is consistent with the tree habit, *Microcycas* has a large ovulate cone (1), but not as large as in some species of *Encephalartos*, *Macrozamia*, and *Dioon*. The cone itself ranks high in the scale of evolution, the sporophylls being very unleaflike and reduced in size. The size of ovules puts *Microcycas* among the more advanced cycads. They are smaller than those of *Cycas*, *Encephalartos*, *Macrozamia*, and *Dioon*, yet larger than those of *Zamia*, *Bowenia*, and *Stangeria*. They are about the same size as those of *Ceratozamia*, although possibly averaging larger.

The female gametophyte is quite similar to the female gametophytes of the other cycads, except in the thickness of the megaspore membrane, and in the number of the archegonia. Comparing the measurements of the megaspore membranes made by THOMSON (16) on five genera of cycads other than *Microcycas*, it would appear that *Microcycas* possesses the thinnest one to start with, and that at its greatest thickness it is approximately the same as that surrounding the free nuclear gametophyte of *Zamia integrifolia* (16), which genus is considered the most advanced of all.

Although the normal appearance of the archegonia is about like that of other cycads, their number is strikingly greater, being sixty-four or more, as compared with the one to ten archegonia

found in the other genera. CALDWELL states that the number sometimes exceeds two hundred, but this number I believe to be unusual, even counting the non-functioning archegonia. Even the least number, however, does not nearly approach the narrow range of the other cycads. This situation might be called primitive. It may be that more cells retain the ability to become archegonia, given the proper stimulus. On the other hand, in view of the situation in *Welwitschia*, this large number might be regarded as an advanced character.

The development of the archegonium is similar to that of *Dioon edule*, described by CHAMBERLAIN (4), with these differences: the archegonial jacket is exhausted two or three months earlier; and a definite ventral canal cell is occasionally formed as in the Ginkgoales (3) and the Abietineae, a more primitive condition than that found in the other cycads, where there is no trace of a wall between the ventral canal nucleus and the nucleus of the egg. The archegonia occur in groups, as in the Cupressineae (13), but have no common archegonial jacket.

Another and strikingly primitive feature of *Microcycas* is the fact that there are sixteen to twenty sperms (1).

Microcycas therefore shows both primitive and advanced characters. It is to be noticed that most of the primitive characters appear in the gametophyte generation, while most of the advanced characters are in the sporophyte generation.

On the whole, *Microcycas* shows more advanced than primitive characters, and should be regarded as one of the more advanced cycads.

Summary

1. The ovules were not young enough to show the megaspore mother cell or the megaspores, the youngest stage of the female gametophyte showing free nuclear condition in the megaspore.

2. In older ovules, nuclear division has increased the number of free nuclei, while at the periphery some of the nuclei are already inclosed by walls. Still older ovules show the gametophyte to be completely cellular.

3. The number of chromosomes is twelve, although occasional nuclei have fourteen.

4. The megaspore membrane increases in thickness from 1.5 μ in August to 3 μ in October, and thereafter decreases to 1.0 μ in March.

5. The cells of the nucellus adjacent to the gametophyte are early organized into a nutritive jacket, which persists until the middle of December.

6. The vascular bundles belonging to the inner fleshy layer of the ovule divide and give rise to branches which enter the nucellus.

7. The archegonial initials are distinguishable about the latter part of November. They are scattered in groups over the surface of the gametophyte, but only those at the micropylar end develop. The remaining initials may divide and become almost unrecognizable among the other gametophyte cells, or may become merely multinucleate.

8. The development of the archegonia is the same as in other cycads, with the exception that in some cases a ventral canal cell is formed.

9. Haustoria from the egg protrude through pores in the egg membrane into the cells of the archegonial jacket which surrounds each archegonium.

10. The archegonial chamber is a shallow depression occurring late in the development of the gametophyte, and is not nearly so highly developed as in the other genera.

11. *Microcycas* should be regarded as one of the more advanced cycads.

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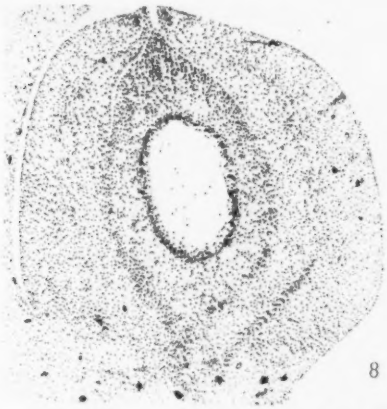
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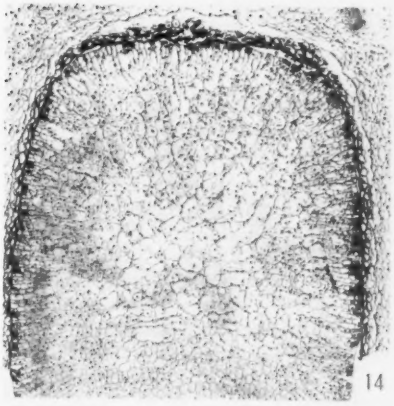
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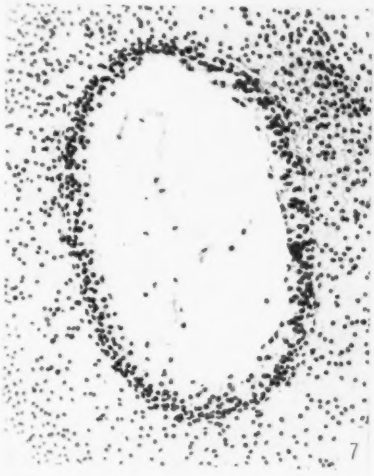
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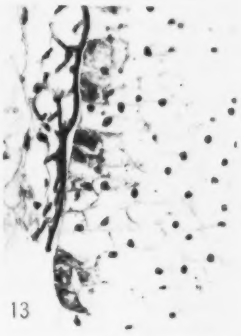
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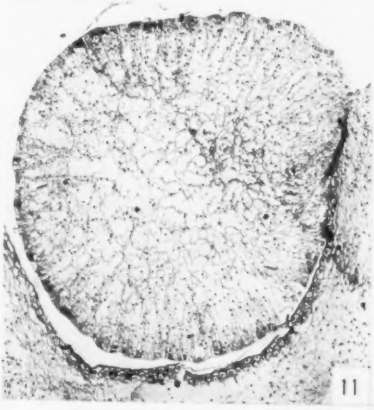
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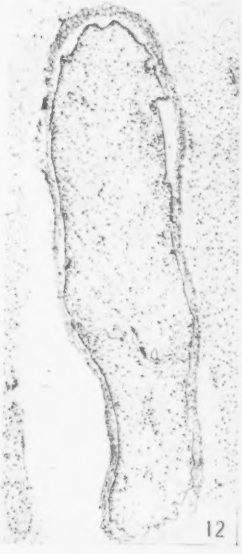
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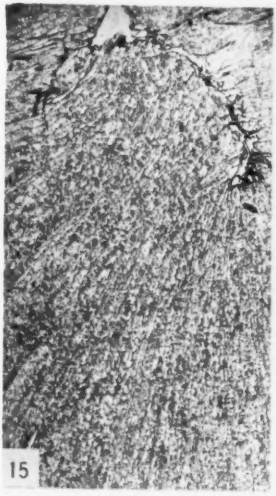
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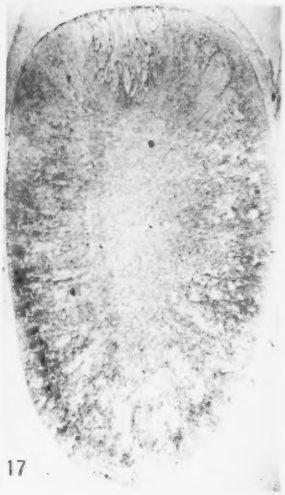
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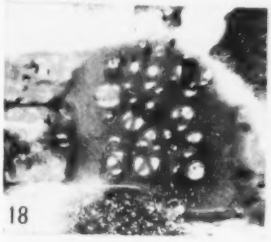
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EXPLANATION OF PLATES XXVIII, XXIX

FIG. 7.—Free nuclear stage of gametophyte with young endosperm jacket; ×67.

FIG. 8.—Young ovule containing free nuclear gametophytes surrounded by endosperm jacket; ×26.

FIG. 9.—Young, completely cellular gametophyte; ×26.

FIG. 10.—Detail of fig. 9, showing endosperm jacket, megaspore membrane, and arrangement of cells of gametophyte in regular rows; ×132.

FIG. 11.—Transverse section of young gametophyte; ×25.

FIG. 12.—Two gametophytes each within its own megaspore membrane, but inclosed by common endosperm jacket; gametophytes same age as one shown in fig. 9; ×26.

FIG. 13.—Detail of young gametophyte; note heavy walled endosperm jacket cells and regular rows of gametophyte cells; ×64.

FIG. 14.—Older gametophyte, showing location of possible archegonial initials; section not perfectly median; ×25.

FIG. 15.—Median section of mature gametophyte, showing cells in rows radiating from suture; ×16.

FIG. 16.—Tangential cut of lower part of mature gametophyte, showing alternation of light areas and dark bands; ×10.

FIG. 17.—General topography of early December gametophyte; ×6.

FIG. 18.—Detail of egg membrane in surface view; note arrangement of pores; ×190.

FIG. 19.—Multinucleate archegonium from February gametophyte; nuclei arisen from division of central cell nucleus before division to form ventral canal nucleus; ×102.

FIG. 20.—General topography of November ovule, showing partial exhaustion of nucellus; ×9.

FIG. 21.—Binucleate central cell resulting from division shown in fig. 22; note also two tiers of neck cells; archegonia of figs 21–23 are from gametophyte of age shown in fig 17; ×308.

FIG. 22.—Young archegonium in which central cell nucleus is divided; ×330.

FIG. 23.—Young archegonium in which nucleus of central cell has assumed a central position; ×308.

QUANTITATIVE RELATIONS OF CARBOHYDRATES TO NITROGEN IN DETERMINING GROWTH RESPONSES IN TOMATO CUTTINGS

MARY E. REID

(WITH EIGHT FIGURES)

The investigations described and results presented in this paper are the outcome of a study of the utilization of the different kinds of food reserves in tomato cuttings in their relation to the character of the growth responses which they display. Variations in external conditions have been used, such as growing the cuttings in light and in darkness, and in solutions with and without nitrate nitrogen.

This problem was suggested for investigation as a result of certain observations of the growth responses of tomato cuttings described by KRAUS and KRAYBILL in their work on vegetation and reproduction with special reference to the tomato.[1]

Pieces of stems one to four inches long, without leaves and possessing both nodes and internodes, were examined microchemically to learn something of the nature of their content. They were then placed on filter paper moistened with distilled water and placed under a bell jar in the laboratory. These trials were repeated several times, always with the same results. (1) Yellowish stems high in carbohydrates and low in total nitrogen and nitrates pushed forth many roots, particularly along the internodes, to the length of one to four inches. One or two formed tiny yellowish sprouts at the nodes. In ten days to two weeks the roots turned dark and began to decay. (2) Greenish stems containing starch and fairly high in total nitrogen always produced roots along the internodes and sometimes small green sprouts at the nodes. The root production was not so profuse as in the foregoing. Decay began in about the same length of time. The succulent tops of the same plants without starch reserves all decayed without root or shoot production. (3) Green, succulent stems, without starch reserves and very low in free reducing substances but high in total nitrogen and nitrate nitrogen, all decayed without root or shoot production. These results are of interest in connection with the vegetative propagation of many plants, for which purpose the practical grower prefers the more "hardened" or mature portions. From the general viewpoint expressed in this paper they are also interesting in connection with some other experiments on tomatoes

[1] KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Exp. Sta. Bull. 149, 1918.

which will not be discussed here, except to state that a decided reduction in the development of the root systems of the plants accompanied a continued removal of leaves from the tops. According to microchemical tests, that practice also resulted in a marked decrease in the carbohydrates in the stems, and a reduction in vegetative extension and fruitfulness.

Only a brief statement will be given in this paper of the results of the writer's investigations, which were begun in the fall of 1920 and which have been continued up to the present time. It is hoped that soon a more complete account of the work may be published, giving numerical data and a survey of the literature on the subject.

The methods employed in conducting these experiments were the same as those described in a previous article.[2] The nutrient solutions were prepared according to the following formulas:

SOLUTION CONTAINING NITRATES

A	B
2 per cent $MgSO_4$	3 per cent $CaCl_2$
2 per cent KH_2PO_4	2 per cent $CaSO_4$
2 per cent KNo_3	4 per cent $Ca(NO_3)_2$

SOLUTION MINUS NITRATES

A	B
2 per cent $Mg SO_4$	4 per cent $CaCl_2$
2 per cent KH_2PO_4	2 per cent $CaSO_4$
1 per cent KCl	

In preparing the solutions for the cuttings, 100 cc. of solution A was made up to one liter in distilled water, and an equal quantity of solution B was made up in the same way. These diluted solutions of A and B were mixed before using. A few drops of a 1 per cent solution of ferric citrate were added to each liter of the solution.

Observations

In the previous paper the text figures were so arranged as to show the effect of differences in composition upon the growth of cuttings under uniform external conditions. In this article the cuttings of uniform composition are shown on one text figure, but a comparison of the effects of differences in composition may be seen by referring to the figures on opposite pages. For example,

[2] REID, MARY E., Relation of the kind of food reserves to regeneration in tomato plants. BOT. GAZ. 77: 103–110. 1924.

figs. 1 and 3 are of basal cuttings of the two types of composition. To obtain an idea of the behavior of cuttings dissimilar in composition but grown under the same external conditions, compare cuttings *A* and *A* in the two figures, *B* and *B*, etc.

Fig. 1 shows basal carbohydrate-high cuttings grown under different external conditions. Cutting *A* was grown in the light in the solution plus nitrates, *B* was grown in the dark in the solution plus nitrates, *C* was grown in the light in the solution minus nitrates, and *D* was grown in the dark in the solution minus nitrates. It may be observed that roots grow abundantly on cuttings kept in light and in darkness in both plus nitrate and minus nitrate solutions. There are striking differences in shoot growth under the different external conditions. There are more shoots and a greater weight of shoots produced in light than in darkness in solutions both plus and minus nitrates, but perhaps the most conspicuous feature is that shoots grow only to a small extent and often not at all on cuttings kept in the solution minus nitrates.

In another series of experiments the cuttings were obtained from plants that had been transplanted into sand at an earlier age than the plants from which the cuttings here shown were taken. The nitrogen content of these plants was presumably less than that of the plants transplanted at the earlier age. The growth responses made by these cuttings is shown in fig. 5 (p. 412). This cutting was high in carbohydrates and was grown in the light in the solution minus nitrates. No shoots were produced on any of the basal cuttings in this series which were grown in the solution minus nitrates, but roots were produced abundantly.

These growth responses of cuttings high in carbohydrates and low in nitrogen indicate that nitrate nitrogen may be utilized in darkness in the building of nitrogenous materials that may be used in growth, but the production of such materials in darkness is much less than occurs in light. The addition of nitrates to the nutrient solution often resulted in twice the amount of growth that occurred on cuttings grown in the solution minus nitrates. The difference in the amount of growth in the two solutions is largely a matter of increased shoot production by the cuttings grown in the solution plus nitrates.

The results obtained with high carbohydrate, low nitrogen cuttings grown in the solution minus nitrates appear to indicate that the nitrogenous reserves in defoliated cuttings are utilized more fully when the cuttings are grown in light than when they are grown in darkness. This difference is largely a matter of difference in shoot growth. It seems that cuttings grown in the light are much more likely to produce shoots.

Fig. 2 shows basal cuttings of highly vegetative plants. Cuttings *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. It may be observed that vegetative cuttings grown in the light produce some shoots, but do not produce roots abundantly. In most cases none is produced until after the cuttings have grown in the light for several weeks.

Fig. 3 shows top cuttings of carbohydrate-high plants. *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. It may be noted that the cuttings grown in the light in the solution plus nitrates developed both roots and shoots abundantly. In darkness, both roots and shoots were very limited in quantity. When grown in the solution minus nitrates, the top cuttings produced, at the end of a growing period of about one month, a relatively greater weight of shoots than did the basal cuttings, but relatively less roots.

Fig. 4 shows top vegetative cuttings. *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. The photograph was taken after the cuttings had been growing in their respective solutions for two weeks. Shoots were produced by cuttings grown in the light about equally well in the two solutions. No roots were developed. Neither roots nor shoots grew in darkness, but a withering of the tips was observable after the cuttings had stood in the dark room for five to seven days.

The results obtained with vegetative cuttings in all the experiments thus far conducted have indicated that the vegetative cuttings having the highest carbohydrate supply are the ones that produce the most new growth in darkness. Those cuttings containing

FIG. 1.—Basal cuttings of carbohydrate-high plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

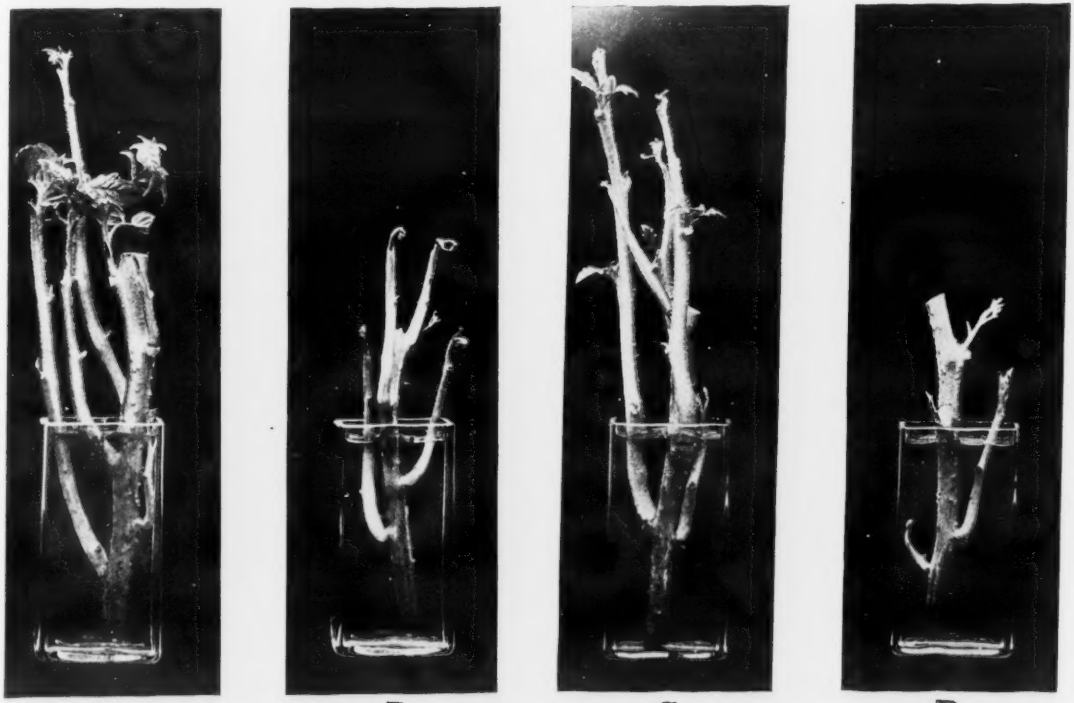

FIG. 2.—Basal cuttings of vegetative plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

FIG. 3.—Top cuttings of carbohydrate-high plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

FIG. 3.—Top cuttings of carbohydrate-high plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

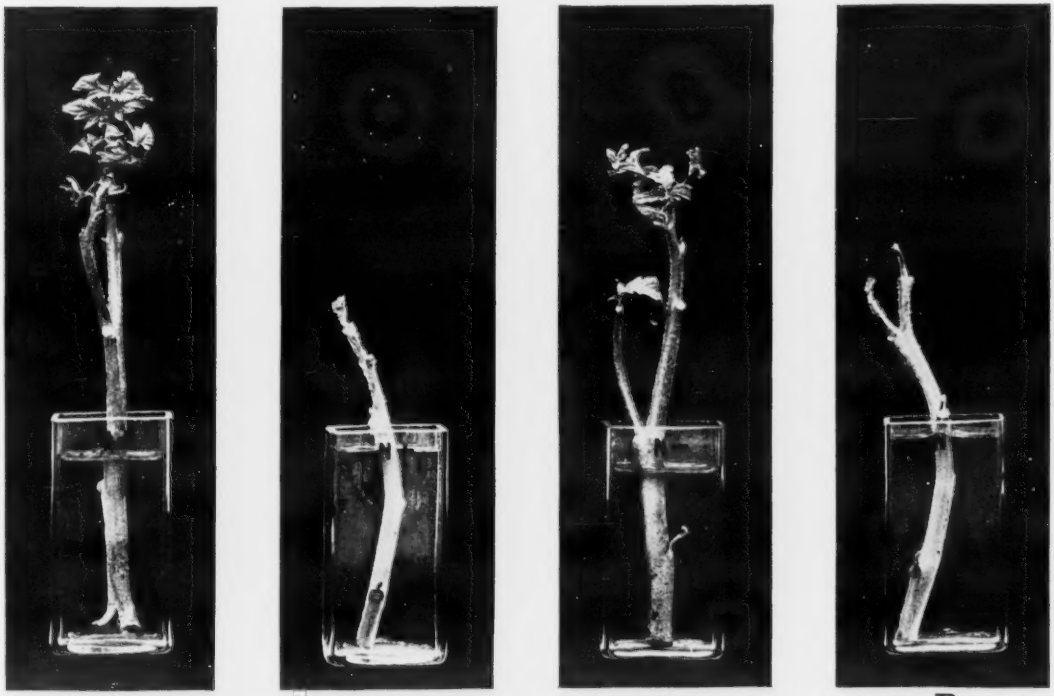

FIG. 4.—Top cuttings of vegetative plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

the greatest amount of young tissue, such as the top cuttings or the basal cuttings with long side shoots, produce the greatest total amount of new shoots when grown in the light. During the first two weeks of the growing period of highly vegetative cuttings, shoots appear to develop equally well in the solutions plus nitrates and minus nitrates. This was true both in light and in darkness. Later, however, those cuttings growing in the light in the solution plus nitrates had the greatest total amount of new shoots.

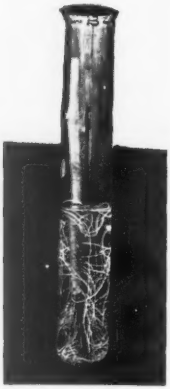

FIG. 5.—Basal cutting of carbohydrate-high plant presumably having lower nitrogen content than cutting shown in fig. 1 *C* (both grown in light in solution lacking nitrates).

Fig. 6 shows top cuttings of vegetative plants grown in the light and grown a week longer than similar cuttings shown in the last figure, in which no roots were present. Cuttings *A–D* inclusive were grown in the solution minus nitrates and *E–H* inclusive in the solution plus nitrates. It has been found in most of the experiments with top vegetative cuttings that, in the first two weeks of the growing period, roots develop in greatest abundance upon cuttings grown in the solution minus nitrates, either in light or in darkness. Later, in the case of cuttings grown in the light in solutions both plus and minus nitrates, the roots appear in equal amounts. Still later, the roots appear to develop more rapidly on the cuttings growing in the solution plus nitrates. This difference between earlier and later periods of growth is believed to be correlated with the larger photosynthesizing area which the cuttings grown in the solution plus nitrates eventually develop. The growth responses appear to indicate that, if the carbohydrate supply becomes the limiting factor, the growth of shoots exceeds that of roots; and that if, on the contrary, the nitrogen supply is the limiting factor, the growth of roots may exceed that of shoots. Considering the growth responses of the highly vegetative cuttings just described, it seems that a large reserve supply of carbohydrates is not directly essential to root growth. Apparently only as the materials enter into solution

FIG. 6.—Top cuttings of vegetative plants grown in light: *A–D*, in solution lacking nitrates; *E–H*, in solution containing nitrates.

do they exert an influence upon the type of growth. At the time these highly vegetative cuttings were made, they contained practically no starch except a few grains in the endodermal cells. After the cuttings developed leaves, carbohydrates were eventually synthesized faster than they were utilized, and there occurred a deposition of starch in typical starch storage cells. An accumulation of carbohydrates occurred earlier in the cuttings grown in the solution minus nitrates than in those grown in the solution plus nitrates. It is not thought that starch itself is the determining factor, but rather that some other conditions accompanying starch deposition may be important, as, for example, a high sugar content.

There appears to be a close relationship between the type of growth response displayed by cuttings and that of the plant from which the cuttings are obtained. The cuttings seem to have a tendency to do what the plant has been doing. If the plants were producing shoots to a small extent but producing roots abundantly, the basal cuttings of such plants tend to produce chiefly roots, and the top cuttings to produce both roots and shoots, the combined effect being a greater total tendency toward root production. If the plant were producing shoots abundantly but roots to a small extent, the basal cuttings produce shoots and a few roots, the tops only shoots, the combined effect being a greater total tendency toward shoot production.

In fig. 7, plant *A* is highly vegetative and of the series of plants from which the vegetative cuttings shown in figs. 2, 4, and 6 were taken. Branches have been produced at most of the nodes, and the leaves are exceedingly large, dark green, and numerous. The root system consists of relatively few, thick, fibrous, short, slightly branched roots. Plant *D* is the carbohydrate-high type from the same series. Its stem is straight and unbranched. The leaves are few, small, and a very light yellowish green. On the other hand, the root system is relatively very large. The roots are very numerous, and, although not long, possess a large surface area in relation to the small size of the plant above ground. The plant shown in illustrations *B* and *C* is not strictly comparable, since it is slightly older than plants *A* and *D*. It is interesting to note that this plant is intermediate in chemical composition, as well as in the relative amounts of roots and shoots.

In fig. 8 are shown iodine-treated sections taken through the basal portion of the stems from the three plants shown in fig. 7. *A* and *B* are sections through the basal portion of the carbohydrate-high plant. The darkened areas in *B* show the heavy deposit of starch in the pith and medullary ray cells. *A* is a section through the same stem, taken through a region in which the starch was not so abundant as in *B*, and in which the individual grains show plainly. Section *D* is through the basal portion of the highly vegetative plant, no starch grains being visible. The tissues shown are the older portion of the xylem in the region of one of the primary vascular bundles, pith cells, and a group of internal phloem cells. Section *C* is through the stem intermediate in type. Considerable starch was present, but very much less than in the carbohydrate-high type of plant.

Discussion

The results of these experiments seem to indicate that the growth responses as expressed in root and shoot production may be determined, at least in part, by the nature of the available food materials. It seems that, if the carbohydrate supply becomes the limiting factor, the growth of tops exceeds that of roots, but that if the nitrogen supply becomes the limiting factor, the growth of roots may exceed that of tops. It seems probable that the relationship must be indirectly between the relative amounts of certain forms of soluble carbohydrates present and the supply of some inorganic form of nitrogen. As a result of the relative proportions of these constituents, organic nitrogen compounds are formed which directly determine the nature of the growth response. It is supposed that a supply of carbohydrates must be present in excess of that used in forming these nitrogenous organic compounds, in order to provide materials for respiration and for building cell walls. It might be assumed as a result of these experiments that merely a difference in the total amount of the organic nitrogen compounds would determine differences in root and shoot growth. Experiments now under way, however, suggest that total nitrogen or total protein content may not explain differences in relative amounts of roots and shoots produced. These same experiments suggest that, given a readily available supply of carbohydrates, it is the balance

FIG. 7.—*A*, highly vegetative plant; *B*, *C*, plant moderately vegetative; *D*, carbohydrate-high plant

FIG. 7.—*A*, highly vegetative plant; *B*, *C*, plant moderately vegetative; *D*, carbohydrate-high plant

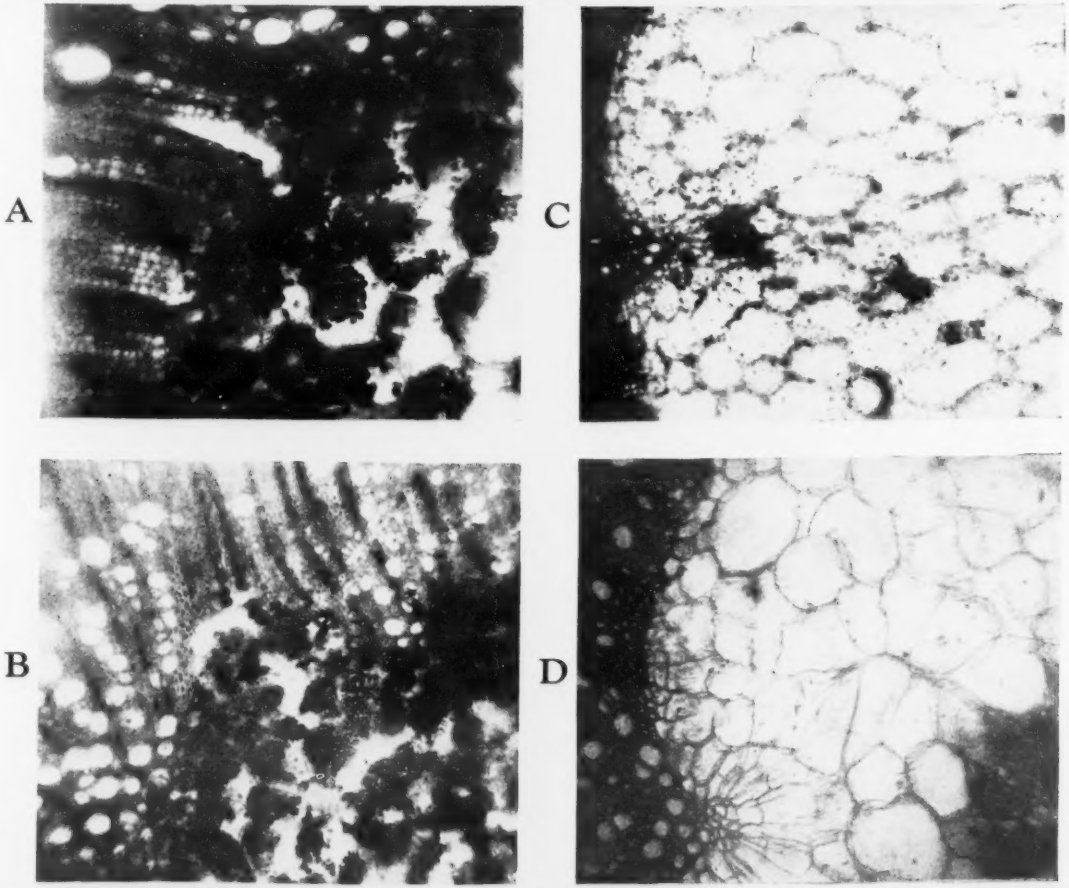

FIG. 8.—Microphotographs of iodine-treated sections taken through basal portions of stems of plants shown in fig. 7, illustrating differences in relative amounts of starch stored: *A*, *B*, sections through stem of carbohydrate-high plant illustrated in fig. 7 *D*; *C*, section through stem of moderately vegetative type of plant shown in fig. 7 *B*, *C*; *D*, section through stem of highly vegetative plant shown in fig. 7 *A*.

that exists between some forms of organic nitrogen that determines the growth responses.

Summary

1. A high nitrogen supply plus a readily available (even though somewhat limited) supply of carbohydrates appears to furnish favorable conditions for shoot growth.

2. A somewhat limited nitrogen supply plus a readily available supply of carbohydrates appears to furnish favorable conditions for root growth.

3. There seems to be a greater utilization of the carbohydrate reserves in carbohydrate-high cuttings grown in light than in those grown in darkness, and the utilization is greater in the cuttings grown in the solution plus nitrates than in those grown in the solution minus nitrates.

4. A synthesis of nitrogenous materials that may be used in growth appears to occur in cuttings grown both in light and in darkness, but such synthesis appears to be greater in amount in light than in darkness.

5. Light is particularly favorable for the growth of shoots.

6. The cuttings have a tendency to do what the plant has been doing. If the plant was producing shoots abundantly and roots to a relatively small extent, the basal cutting from such a plant tends to produce a few roots and some shoots, and the top cutting chiefly shoots, the combined effect being a greater total tendency toward shoot production. If the plant was producing roots abundantly and shoots to a small extent, the basal cutting tends to produce only roots, the top cutting to produce roots and some shoots; the combined effect being a greater total tendency toward root production.

These investigations were conducted in the laboratory of plant physiology of the department of botany at the University of Wisconsin, and were begun at the suggestion of Professor E. J. KRAUS. The writer wishes to extend grateful acknowledgment to Professors J. B. OVERTON and E. J. KRAUS for helpful suggestions and criticisms given during the progress of the investigations.

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EFFECT OF POTASSIUM ACID PHTHALATE ON EARLY GROWTH OF TOMATO PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 319

R. B. DUSTMAN

(WITH SIX FIGURES)

In connection with a study of the effect of reaction values of nutrient cultures upon certain internal factors in plant metabolism, it became desirable to know the effect, if any, of the organic compound, potassium acid phthalate, upon the early growth and vigor of plants subjected to its action. So far as the writer is aware, the use of phthalic acid or its salts in nutrient cultures has been reported from a single source, the work of TARR and NOBLE (**6**). These investigators employed potassium acid phthalate for the purpose of stabilizing the hydrogen ion concentrations of their nutrient solutions. They report excellent buffer action from its use, but state that they were unable to determine definitely whether or not it exerted a harmful effect upon the plants grown. The results were regarded as being indicative of no such effect. It is known, however, that many of the benzene ring derivatives may exert a toxic influence upon growth. Thus BRENCHLEY (**1**) found an inhibitory action from various phenols, all of which produced the same general effect, but which varied considerably in respect to the concentrations at which these effects became apparent. SCHREINER and SKINNER (**5**) showed that relatively small amounts of salicylic aldehyde were toxic to the growth of various plants in water cultures and in pot and field experiments. Unpublished data from this laboratory indicate that benzidine is quite harmful to the growth of soy beans and barley. Unfortunately none of these compounds is sufficiently closely related to the one in question to permit comparison. For these reasons five series of experiments were arranged and carried out in a small but carefully conducted manner.

SERIES I.—Sand cultures in half gallon jars; five lots including 000, 1000, 2000, 4000, and 10,000 ppm of phthalate; T and N

nutrient solution; P_H range 5.03–5.05; three plants per jar; variety Bonny Best.

SERIES II.—Sand and water cultures in glass stenders; six lots of each including 000, 500, 1000, 1500, 2000, and 2500 ppm of phthalate; T and N nutrient solution; P_H range 5.02–5.07; three plants per stender; variety Bonny Best.

SERIES III.—Sand cultures in half gallon jars; six lots including 000, 500, 1000, 1500, 2000, and 2500 ppm of phthalate; NRC Type III R_6S_1, nutrient solution; P_H range 3.73–3.83; three plants per jar; variety Bonny Best.

SERIES IV.—Sand cultures in glass stenders; six lots each of barley and tomatoes including 000, 200, 400, 600, 800, and 1000 ppm of phthalate; T and N nutrient solution; P_H range 5.02–5.06; four plants per stender; varieties Wisconsin pedigreed barley and Stone tomatoes.

SERIES V.—Soil cultures in half gallon jars; six lots including 000, 125, 250, 500, 750, and 1000 parts of phthalate ppm of soil; five plants per jar; variety Stone.

Methods

All experiments were run in duplicate. The plants were grown under greenhouse conditions and consisted, in all instances but one, of tomatoes of the Bonny Best and Stone varieties. The single exception was a parallel set of pure line barley cultures grown as a check against the tomatoes in the series containing the lowest concentrations of phthalate. Water, sand, and soil cultures were employed, but the former proved unsatisfactory. Most of the cultures were of sand media arranged partly in large glass stenders of 250 cc. capacity, and partly in half gallon jars of glazed earthenware. The glass containers were wrapped with black glazed paper to protect the roots from the action of light. In all, ranges of 200–10,000 ppm of phthalate were tried.

Two types of nutrient solutions were employed, including the one used by TARR and NOBLE (**6**) and the National Research Council (**4**) type III, row 6, solution 1. The latter was chosen because of its reported hydrogen ion concentration, which according to MCCALL and HAAG (**3**) is P_H 3.9 by the colorimetric method,

but which as made up and measured in these experiments gave a reaction value of P_H 3.73 with the Leeds and Northrup Type K potentiometer, equipped with a modified HILDEBRAND (**2**) type of hydrogen electrode and a saturated calomel electrode with liquid junctions across saturated KCl solution. This instrument was used in adjusting the various nutrient solutions of the sand and water cultures to a uniform value of approximately 5 P_H by the addition of tenth normal sodium hydroxide. The actual range of all the nutrient cultures in their initial reaction values was P_H 5.02–5.07, except the National Research Council type III solutions, which were taken at their resulting values of P_H 3.73–3.83 after addition of the varying increments of phthalate and without adjustment with sodium hydroxide.

The sand used was from a fresh supply of pure white quartz of high quality and medium texture (no. 3 grade) prepared by the Wausau Abrasive Company, Wausau, Wisconsin. It was used directly without washing or other previous treatment. The chemicals were of standard purity as supplied by the Baker Chemical Company and the Central Scientific Company of Philadelphia and Chicago, respectively. The soil used was a rich potting soil from the greenhouse, heavily manured but otherwise unfertilized. Its reaction value was undetermined, but could not well have been very acid, as it contained numerous small shells of carbonate of lime. In preparing the pots the dry soil was spread out and the phthalate applied in solution. After thorough mixing the soil was returned to the jars.

In the series containing the water cultures these were equipped with paraffin mounts with small holes through which the previously germinated seedlings were thrust into the solution beneath.

No applications of either nutrient materials or potassium acid phthalate were made subsequent to the initial application in any of the experiments, and no attempt was made to control reaction values after the seeds were planted. Waterings were made with distilled water alone, the stender cultures receiving equal volumes until differences in growth necessitated adjustment in accordance with transpiration, whereas the pot cultures were held at uniform moisture content by frequent, usually daily, weighings. Growth

periods ranged from twenty to thirty-five days, except for the water cultures, which were discontinued after two weeks.

Data

Series I was set up and planted March 8 with ten seeds per pot, at which time was applied also 500 cc. of the T and N adjusted nutrient solution. The plants were later thinned to three per jar. Table I shows the results secured.

Series II (including both sand and water cultures) was started April 10 and 11. The water cultures were supplied with 200 cc. of adjusted T and N solution and three seedlings each. By the end of the second week it was apparent that these cultures were unreliable. They therefore were discontinued. Several of the solutions were showing contamination with mold and algal growth,

TABLE I

EFFECT OF STRONG CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 35 DAYS)

Lot no.	KH phthalate ppm	N/10 NaOH added per liter (cc.)	Initial P_H	Final dry weight per plant (gm.)	Final P_H
1.....	None	86	5.05	0.0759	3.85
2.....	1000	110	5.04	0.0707	6.64
3.....	2000	135	5.04	0.0174	7.46
4.....	4000	180	5.03	Dead	.....
5.....	10,000	330	5.05	Dead	.....

and there was some indication that the paraffin injured the seed leaves where they rested upon it, the sunlight and temperature being sufficient to soften and slightly melt the surface of the mounts. The sand cultures of this series proved to be much more satisfactory. They were treated with 90 cc. of T and N adjusted nutrient solution, planted with five sprouting seeds each, and four days later thinned to three seedlings each. The results are shown in table II.

Series III contained the same concentrations of phthalate as Series II, but was based upon a totally different nutrient solution. As already stated, the National Research Council type III R_6S_1 was chosen because of its reported reaction value of P_H 3.9. As made up and used with the addition of phthalate and without adjustment with NaOH, the initial range of reaction values was

P_H 3.73–3.83. The half gallon jars of sand were planted April 14 with seven previously soaked seeds in each, at which time 500 cc. of the solution was applied. These were later thinned to three plants per jar. Table III shows the yields secured.

Series IV was carried out in stenders and included parallel sets of tomatoes and barley. The solutions for both were made up

TABLE II

EFFECT OF MODERATE CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 20 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NaOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm.	Dry gm.
1.....	None	86	5.06	1.0043	0.0958
2.....	500	100	5.07	0.8734	0.0786
3.....	1000	110	5.02	0.5006	0.0449
4.....	1500	125	5.04	0.4236	0.0302
5.....	2000	135	5.06	0.2858	0.0280
6.....	2500	145	5.05	0.2536	0.0263

TABLE III

EFFECT OF MODERATE CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 35 DAYS)

LOT NO.	KH PHTHALATE PPM	P_H	AVERAGE WEIGHT PER PLANT	
			Green gm.	Dry gm.
1.....	None	3.73	4.3087	0.4317
2.....	500	.....	2.7402	0.2995
3.....	1000	.....	2.0531	0.1887
4.....	1500	.....	1.2359	0.1082
5.....	2000	.....	1.3660	0.1270
6.....	2500	3.83	0.3607	0.0411

together and divided between the two sets. On April 27 the cultures were set up with 100 cc. of T and N adjusted solution and planted with seven barley grains or ten tomato seeds each. When well up the plants were thinned to four per culture. The barley plants were harvested May 19 and the tomatoes May 24. The yields are shown in tables IV*a* and IV*b*.

Series V included the single group of soil cultures tried. The jars were planted April 28 with twelve seeds each, and a week later thinned to five plants per jar. They grew vigorously and were harvested May 24. The results are shown in table V.

TABLE IV*a*

EFFECT OF WEAKER CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 27 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NaOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm.	Dry gm.
1.....	None	86	5.06	2.2919	0.2304
2.....	200	93	5.05	2.0042	0.2074
3.....	400	97	5.04	1.8822	0.1964
4.....	600	101	5.03	1.7427	0.1785
5.....	800	106	5.04	1.7403	0.1938
6.....	1000	110	5.02	1.5414	0.1761

TABLE IV*b*

EFFECT OF WEAKER CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF BARLEY PLANTS (GROWTH PERIOD 22 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NaOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm.	Dry gm.
1.....	None	86	5.06	1.0455	0.1492
2.....	200	93	5.05	0.9998	0.1274
3.....	400	97	5.04	0.7595	0.1056
4.....	600	101	5.03	0.5433	0.0758
5.....	800	106	5.04	0.7327	0.0951
6.....	1000	110	5.02	0.6473	0.0893

Discussion

The general course of development in these experiments was from stronger to weaker concentrations of phthalate. As soon as it became apparent that a given series was showing a harmful effect from the phthalate added, a new series in which the concentration was reduced was put under way. The primary object was to determine whether or not the phthalate exerted a toxic action on tomato plants, in concentrations suitable for buffering the solutions in which they were grown. Consequently its effect in concentra-

tions below 200 ppm was not studied. TARR and NOBLE had already shown that it exerts a good buffer action at a concentration of 1020 ppm in the solution employed by them. It was thought that perhaps even greater concentrations could be used without harmful results, thus necessitating less frequent changes of solutions, and still maintain constant reaction values. The outcome of the trials herein reported not only demonstrates the fallacy of this view, but raises a serious question as to the freedom of the solutions, from toxic influences when containing potassium acid phthalate in concentrations considerably below 1000 ppm.

TABLE V

EFFECT OF KH PHTHALATE ON TOMATO PLANTS GROWN IN RICH SOIL (GROWTH PERIOD 27 DAYS)

LOT NO.	PARTS OF KH PHTHALATE PPM OF SOIL	AVERAGE WEIGHT PER PLANT (TOP ONLY)	
		Green gm.	Dry gm.
1	None	16.38	1.115
2	125	17.40	1.279
3	250	18.20	1.269
4	500	18.74	1.383
5	750	18.24	1.272
6	1000	19.00	1.509

Table I shows that the seedling tomato plants were unable to endure the stronger concentrations. In this connection it should be stated that the very much increased osmotic concentration incident to the additions of phthalate and sodium hydroxide probably contributed materially to their failure. In fact, there were several variables in this work whose partial effects were difficult to separate and evaluate, especially in the higher concentrations. These were the potassium acid phthalate, sodium hydroxide, total osmotic concentration, and reaction value. The three last mentioned are believed to have been eliminated, so far as harmful effects are concerned, in the later experiments embracing the lesser concentrations of phthalate and reduced total osmotic values.

There are certain inherent difficulties involved in the use of culture solutions where concentration of hydrogen ion is an important

feature in the studies being made. No data are known to the writer which might suggest the reaction value most favorable for the growth of tomato plants. The fact, however, that most plants tried have shown satisfactory growth from P_H 4 to 6, and a general knowledge of the tomato as applied to its growth under field conditions, would lead one to believe that it is not particularly sensitive to moderately acid conditions, and that these values would permit normal development. Accordingly, a P_H value of five was arbitrarily chosen for the majority of the solutions, although they probably did not remain continuously at this value.

A comparison of tables II and III indicates that the phthalate is the important factor entering into the reduction of growth, since it is active alike at P_H 3.7 and 5.00, and in solutions of dissimilar composition. This comparison also eliminates the sodium hydroxide and sodium ion from consideration at these concentrations, since the former was not added in series III, and no sodium salt entered into the composition of the solution.

From series IV it is apparent that the toxic action of the phthalate is not specific for tomatoes, as it caused a somewhat greater relative reduction with barley than with tomatoes. The yields are somewhat irregular here, as elsewhere in the experiments, but some inconsistency is to be expected where the increments of change are small. The general trend of the effect is obvious throughout. In each of the first four series the effect of the phthalate was manifest not alone by growth differences. With increasing concentration its action was reflected in the color, appearance, and general vigor of the plants. The barley plants developed a chlorotic condition roughly proportional to the amount of phthalate present in solution. Photosynthetic disturbances were more apparent with the barley than with the tomato plants. In the higher concentrations, the tomatoes showed some tendency toward malformation of leaves and a very much greater susceptibility to damping off in the seedling stage. When the roots were washed out it was found that their development was more shallow in the stronger concentration of phthalate. In general the extent of the root development was about proportional to the top growth, but even when considerably developed laterally they frequently failed to penetrate deeply into the sand.

There were several reasons for not repeating the treatments or changing the solutions during the growth period. Uncertainties as to osmotic relations, reaction values, and parallel treatments of duplicates militated against such a procedure. That changes occurred from those conditions originally present in the solutions

FIG. 1.—Series II: showing decreased growth with increasing phthalate treatment 20 days after planting; range 0–2500 ppm.

FIG. 2.—Series III: same phthalate treatment as in fig. 1, but with different nutrient solution; 35 days after planting.

is almost a certainty. The extent of such changes is for the most part unknown. Nevertheless, if an initial application of potassium acid phthalate results in a toxic action, it seems reasonably certain that its continued presence in the original concentration would not be less harmful. Figs. 1–6 show the plants of the various series

at the time of harvest, and give some idea of the relative growths secured.

Series V presents an interesting reversal of the results secured from the sand and water cultures. It was anticipated that the

FIG. 3.—Series IV: effect of less concentrations of phthalate on tomatoes 27 days from planting; range 0–1000 ppm.

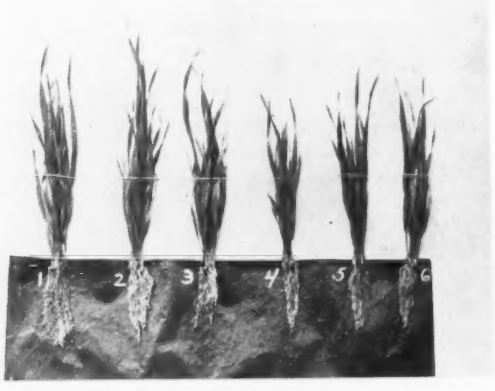

FIG. 4.—Series IV: same treatment as in fig. 3 on barley 22 days from planting

toxic effect of the phthalate would be less pronounced in the soil cultures. That the compound might actually show a positive beneficial effect was entirely unexpected. The close agreement of the duplicates at the two extremes of the series, although not evident from the table, leaves little room for doubt as to its action. In

searching for an explanation for this effect, two possibilities suggest themselves: (1) the phthalate ion may have been removed from solution by adsorption; or (2) it may have been more rapidly attacked and broken down by the much more numerous mold and bacterial flora of the soil. Of the two, the former would seem to be

FIG. 5.—Series V: showing increased growth with increasing phthalate treatment on soil cultures 27 days after planting; range 0–1000 ppm.

FIG. 6.—Series V: same as fig. 5, showing duplicate pots in lots 1 and 6

the more likely explanation, as no differences were apparent during the first fifteen to eighteen days of growth. Presumably the potassium component of the phthalate was responsible for the increased growth. The outcome of this experiment might serve as an illustration of the dangers involved in a too general application of observed phenomena or results secured under specific conditions to a parallel but somewhat different situation.

Summary

A study has been made of the effect of varying concentrations of potassium acid phthalate on the early growth of tomato plants. Culture experiments were carried out under greenhouse conditions, and included water, sand, and soil media. In all, ranges of 200 to 10,000 ppm of the phthalate were tried. With one exception the nutrient solutions of the sand and water cultures were adjusted to a uniform reaction value of 5 P_H by the addition of tenth-normal sodium hydroxide. In this one instance a nutrient solution of different type was employed. Its reaction value was left undisturbed at approximately 3.75 P_H. No attempt was made to control reaction values after the seeds were planted, and no subsequent applications of nutrients or phthalate were made. All experiments were run in duplicate, the averages of which are reported in tables I–V. The plants were watered with distilled water, and the growth period ranged from twenty to thirty-five days.

Conclusions

1. Potassium acid phthalate in concentrations of 1000 or more ppm is decidedly harmful to young tomato plants grown in sand and water cultures.

2. As measured by dry weight increases of plants grown in sand cultures, its toxic action is appreciable in concentrations of 500 ppm or less.

3. When applied to soil cultures the opposite effect may occur, and the addition of the phthalate prove beneficial to the growth of the plants.

4. Barley plants give evidence of being somewhat more susceptible to phthalate injury than tomato plants.

The writer desires this opportunity to express his indebtedness to Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany for continued interest and helpful advice throughout the course of the experiments.

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FACTORS GOVERNING SEASONAL CHANGES IN TRANSPIRATION OF ENCELIA FARINOSA

EDITH B. SHREVE

In a previous paper[1] it has been shown that the desert perennial *Encelia farinosa* possesses some means of reducing its ratio of transpiration to evaporation (T/E) during the months in which aridity is increasing in its native habitat in southern Arizona. Attention has been called to the fact that the plant thus obeys LE CHATELIER's theorem.[2] The evaporative power of the air is twice as great during May and June as during the cool and more humid months of January and February, while *Encelia* loses only 1.4 times as much water per unit area in the latter months as it does in the former. This investigation was undertaken with the hope of discovering the mechanism of the reduction in relative transpiration.

The plant has two distinct types of leaves, a mesophytic form which is present during the cool months, and a smaller xerophytic one which follows the disappearance of the other form. The mesophytic form is sometimes almost glabrous, while the xerophytic form is always covered with a thick mat of hairs, shown in the illustrations in my previous paper. In order to ascertain whether or not the leaf tissue alone accounts for the greater resistance to water loss by the xerophytic form, disks were cut from corresponding positions in the two types of leaves, and the water loss measured under the same external conditions. Samples were always taken just before sunrise.

A combination of air temperature and humidity was selected, lying about midway between the two seasonal extremes, and these conditions were maintained as closely as possible in a closed glass

[1] SHREVE, EDITH B., Seasonal changes in the water relations of desert plants. Ecology **4**: 266–292. 1923.

[2] The theorem is stated by PULLING as follows: "Each change in an outer condition that affects a body or system produces in it a change in such a direction that as a result of this change the resistance of the body or system to this outer change is increased."

chamber. An apparatus was designed for exposing both sides of the disks to the air in such a way that evaporation from the cut surfaces was avoided. Circular holes smaller than the disks were cut at regular intervals in rectangular pieces of aluminum sheeting; the leaf disks were placed over the holes between two sheets and the sheets screwed together. These disk mountings were then arranged so that both sides of the disks were exposed to the air in a vertical position. While weighings were being made, an air-tight cover was fitted over the mountings to prevent loss of water. Eight mountings, each containing eight disks, were then arranged with equal spacing in a glass chamber. Other similar pieces of apparatus filled with wet gray plant drying paper and exposed in a similar manner provided a check on the evaporative conditions of the chamber at all times. It is to be noted that the water loss from the leaf disks was not true transpiration as it occurs in nature. The influence of the water relations of stems and roots has been eliminated, and thus it is possible to compare directly the resistance to the evaporative power of the air offered by the different types of leaf tissue.

In table I are given typical results from these experiments. The figures represent averages of eight mountings, the error due to averages being 0.0005. It is seen that when disks of the two types are placed under the same atmospheric conditions, the xerophytic type loses 0.52 times as much as the mesophytic, a result which seems in agreement with the one that might have been expected from an inspection of the leaf structure. An examination of the water contents of the disks on the two dates, however, shows that there is another factor to be considered. On March 14 the water content of the mesophytic type was 3.3 gm. per gram of dry weight, and the xerophytic type on May 14 was only 1.2 gm. On March 27 the mesophytic leaves were still on the bushes, but their water content had decreased to 2.3 gm. The water loss on March 27 was 0.84 that of the earlier date.

In order to find the effect of a higher water content on the evaporation from the xerophytic disks, branches were cut from the bushes, and the ends immediately placed in water. The branches were covered with bell jars and left to absorb water for 24 hours.

TABLE I

AVERAGES OF RESULTS FROM EXPERIMENTS WITH DISKS OF *Encelia* LEAVES PLACED UNDER IDENTICAL EXTERNAL CONDITIONS; 324 DISKS, 1.6 CM. IN DIAMETER, USED ON EACH DATE

Dates and notes	1	2	3	4	5	6	7	8	9	10	11	12
	Original water content per gm. dry weight (entire leaves)	E_{d} Water loss from 18 disks (leaves)	E_{a} Water loss from 18 disks (paper)	O Original water in 18 disks	M Maximum water in 18 disks after imbibition	Water deficit	Original dry weight 18 disks	Dry weight 18 disks after imbibition	Soluble material lost by imbibition	$E_{d} \times \frac{M}{O}$	$\frac{E_{d}}{E_{a}} \times \frac{M}{O}$	Temperature of cage (F°)
March 14 Mesophytic leaves	3.3	0.143 (1.00)	0.80	0.735	1.08	0.29	0.22	0.16	0.06	0.210	0.262	81
March 27 Mesophytic leaves	2.3	0.120 (0.84)	0.80	0.635	1.12	0.35	0.28	0.15	0.13	0.212	0.265	81
May 14 Xerophytic leaves	1.2	0.075 (0.52)	0.80	0.590	1.67	0.92	0.41	0.25	0.16	0.212	0.265	81
May 18 Xerophytic leaves from branches that have absorbed water for 24 hours	2.3	0.113 (0.79)	0.80	0.685	.....	.....	0.31	.....	.....	.....	.....	81
May 27 Xerophytic leaves from plants irrigated for 10 days	2.0	0.105 (0.74)	0.80	0.675	1.37	0.61	0.34	0.25	0.09	0.213	0.266	81

Disks were then cut from these leaves and their water loss measured in a manner identical with that used for the untreated leaves. The water loss returned to an amount 0.79 as great as that from the mesophytic leaves on March 14. The water content of these xerophytic leaves had increased from 1.2 to 2.3 gm. The water content of other xerophytic leaves was increased from 1.2 to 2.0 gm. by irrigating for ten days plants growing naturally in the open. Again the water loss for equal areas increased markedly (table I). Thus it is evident that the leaf structure cannot be the main factor in increasing the resistance to water loss by evaporation. The evidence of this experiment is that the water deficit in the leaf is a strong controlling factor. When an attempt to obtain a constant is made by using the water loss for equal areas and the water content based on dry weight the result is negative. If the water loss for equal areas is divided by the actual amount of water in the same areas, an approach to a constant is obtained from the ratios E_d/O, but it is far enough away from a true constant to make it certain that other factors must operate.

Working on the hypothesis that a relation exists between the water absorbing capacity and the water holding ability or retentiveness of leaf tissue, the imbibition of leaf disks for both seasons was measured. The two sets of experiments of course were conducted at the same temperature. The maximum water absorbed by equal areas is given in column 5. Here again there is very evidently some relation, for the greater the absorbing capacity the less the water loss by evaporation; but no true constant appears. If, however, the two factors (original water and maximum water obtained by imbibition) are considered together in the form $E_d \times M/O$, a constant is obtained which is exceptionally good for plant work. This is especially true when it is observed that the third decimal is uncertain. It thus appears that under identical atmospheric conditions the water loss from the leaf tissue of this plant varies directly with the original amount of water present, and inversely with the water absorbing capacity of the tissue, regardless of whether the leaf is of the xerophytic or the mesophytic type.

Further evidence of this relation appears in some preliminary experiments that were originally discarded, either because porous

cup atmometers were used instead of felt paper, or because the cage failed to hold a constant temperature. The results of these experiments are given in table II. The water losses from leaf disks at the different temperatures have been reduced to comparable terms by using the response to the evaporative power of the air, E_d/E_a, after the manner of the well known transpiring power ratio T/E. On March 1 and 12 the disks used were of a larger diameter; and cylindrical porous cup atmometers measured the evaporative power of the air. The expression $E_d/E_a \times M/O$ gives the same number for the two dates. On the remaining three dates given in table II the disks were of the same diameter as those in table I, and felt paper measured the evaporative power of the air. Again a constant for these dates appears. If the ratio E_d/E_a is used instead of E_d in table I, the numbers obtained from the expression $E_d/E_a \times M/O$ are the same for all dates with the exception of the first two in table II. These last could not agree with the others because the atmometers give a different unit for the evaporative power of the air; but it is significant that they agree with each other. The expression may now be stated thus: the response to the evaporative power of the air shown by isolated pieces of the leaf tissue varies directly with the original water content of the tissue and inversely with the maximum water content obtained by its imbibition of water in vitro.

It must be remembered that this expression represents the relations existing in an isolated piece of leaf tissue which cannot draw water from the rest of the plant. In the entire plant system the complications of the water content of stems and roots and their water absorbing and retaining capacity, as well as the resistance of water passage, enter into the final resultant of the drought resistance of the whole plant. These experiments show the internal factors operating in the leaf tissue alone. In my former paper it was shown that the transpiration per unit area for a 24 hour period for *Encelia* (T) is a function of at least two external factors, the evaporation power of the air (E) and the water content of the soil (S). The expression $T/(E \times S)$ so nearly approached a constant value that the conclusion was drawn that these two factors represented the major external influences regulating transpiration. Attention was called to the fact, however, that the results showed that still other condi-

tions must be operative, and that these were most probably internal. The ratio of the evaporative power of the air to soil water content (E/S) affects the water content of leaf tissue, which in turn affects the water absorbing capacity of the tissue. Thus the two expressions $T/(E \times S)$ and $E_d/E_a \times M/O$ partly indicate the resistance of *Encelia* to drought and its accord with LECHATELIER'S theorem.

The changes in original water content of leaf tissue seem to be accounted for by the failure of the plant to maintain its water

TABLE II

AVERAGES OF EXPERIMENTS WITH DISKS OF *Encelia* LEAVES UNDER SLIGHTLY DIFFERENT EXTERNAL CONDITIONS

Dates and notes	1 E_d Water loss per hour (leaf disks)	2 E_a Water loss per hour (atmometer)	3 O Original water in 18 disks	4 M Maximum water in 18 disks	5 E_d/E_a	6 $E_d/E_a \times M/O$	7 Diameter disks (cm.)*
March 1 Mesophytic leaves first 2 hours	0.16	0.64	0.94	1.25	0.250	0.332	2.6
March 12 Mesophytic leaves first 2 hours	0.18	0.83	0.87	1.34	0.217	0.333	2.6
March 12 Mesophytic leaves first hour	0.125	0.69	0.735	1.08	0.181	0.265	1.6
May 13 Xerophytic leaves first hour	0.074	0.78	0.590	1.67	0.095	0.268	1.6
May 28 Xerophytic leaves first hour	0.120	0.91	0.675	1.37	0.132	0.268	1.6

* Area exposed to air same in all cases.

balance with increasing aridity; but the causes for the changes in absorbing capacity are evidently more difficult to determine. An examination of the values obtained for the dry weights before and after imbibition throws some light on the mechanism of the changes in water absorbing capacity. The dry weights for eighteen disks, that is, for equal areas, vary considerably with the conditions of the experiments, the greatest difference being between March 14 and May 14 when the xerophytic disks have a value nearly 1.9 greater than the mesophytic.

When these disks were left in distilled water for 24 hours, soluble material diffused into the water; but the amounts were different for the different dates. The less the original water content of the leaf the greater the amount of soluble material that diffused into the water. The dry weights of the disks after imbibition show one constant value for the mesophytic form and a larger one for the xerophytic. In the xerophytic leaf the palisade cells are shorter and more closely packed than in the mesophytic, where there are large intercellular spaces. Thus disks of equal areas would include more cells and consequently more insoluble material than the mesophytic disks, and the difference of 0.15 and 0.25 in the dry weights might be accounted for.

The difference in the diffusible material offers an interesting hypothesis. It may be supposed that, as the plant loses water by excessive transpiration, reversible chemical changes occur that cause certain materials to give up water of combination and to become temporarily insoluble. When more water is available, these substances again undergo change into a soluble form, and are carried by diffusion into other parts of the plant. The results of these experiments agree with this hypothesis. When the plants with xerophytic leaves were irrigated, the original weight decreased from 0.41 to 0.34 gm., and when the cut branches were left in water for 24 hours, the decrease was from 0.41 to 0.31 gm.; while natural desiccation of the mesophytic leaves from 3.3 to 2.2 gm. was accompanied by an increase in dry weight from 0.22 to 0.28 gm. Thus it seems highly probable that the increase in dry weight for equal areas which accompanies a decrease in the water content is the cause of the increase in the water absorbing capacity.

In times of drought the leaves and stems are filled with a dark brown, viscous sap which is almost unfilterable, and which is probably a colloid solution. This liquid oozes from every wound under drought conditions, but is absent during the moist season. When the brown sap is expressed from the leaves and heated to 80° C., a bulky gelatinous precipitate is thrown down. Possibly this contains the material which is rendered insoluble by loss of water, and which is responsible for the increase in water absorbing capacity shown by the tissue of low water content. The amount of precipi-

tate obtained from xerophytic leaves taken from irrigated plants was less than that obtained from the xerophytic leaves of lower water content, a fact in agreement with the theory suggested.

Summary

1. In a former paper it has been shown that *Encelia farinosa*, a desert perennial having a mesophytic form of leaf in the cool moist months and a xerophytic form in the arid season, reduces its response to the maximum evaporate power of the air in June to about one-fifth the January value.

2. The difference in the anatomical structure of the mesophytic and xerophytic leaves does not account for the greater resistance to water loss in the arid season.

3. When disks of equal areas are cut from the two types of leaves immediately before sunrise and placed under identical external conditions, the water loss from them is found to vary inversely with the total imbibitional capacity and directly with the original water content. Even though the external temperature varies as much as 10° F., the expression $E_d/E_a \times M/O$ gives a value which approaches a constant to an amount remarkable for work with water relations of plants.

4. The total imbibitional capacity of both types of leaves varies with the original water content and the dry weight.

5. The lower the original water content, the greater the amount of soluble material that diffuses into the water which surrounds imbibing disks. The dry weights after imbibition have one constant value for mesophytic leaves and another for xerophytic ones.

6. The ratio of evaporation to soil moisture affects the water content of leaf tissue; this in turn affects the imbibitional capacity and resistance to water loss of leaf tissue. The changes in imbibitional capacity are accompanied by changes in the amount of soluble material in the tissue. These changes in diffusible material are presumably the resultants of reversible chemical changes which take up or give up water according to the amount available.

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DEVELOPMENT OF SEED OF CROTALARIA SAGITTALIS

M. T. Cook

(WITH PLATES XXX, XXXI)

This plant was selected for study as typical of those plants in which the embryo absorbs practically all the endosperm and nucellus, and completely fills the one seed coat at maturity. The mature seed, therefore, is composed of a very large embryo and single seed coat. The food for the early support of the seedling is stored in the two very fleshy cotyledons. The seed is of the same type as the bean, but is smaller and much more easily handled for a study of this kind. None of the material collected was young enough to show the development of the embryo sac. The youngest stage observed was an eight-celled sac in which the antipodals were disintegrating and the polar nuclei uniting (figs. 1, 2). It is well known that in the members of this family already studied the antipodals disintegrate very early. It is safe to assume that the development of the embryo sac in this species is in line with that of other species of the Leguminosae. Immediately following fertilization, both the seed and the embryo sac enlarge very rapidly, and the former feels much like a small bladder when pressed between the thumb and finger. This is the result of the very rapid formation of nucellus and its equally rapid disintegration to form the endosperm, which is evidently active in the feeding of the embryo. During this period the sac is lined with a well defined endosperm, and the center is probably filled with liquid food which serves for the immediate use of the growing embryo. The seed, therefore, is almost full size while the embryo is very young and small (figs. 3, 13, 30). The ovules are attached alternately on either side of a ridge along the dorsal side of the ovary (figs. 4, 5); that is, the side corresponding to the midrib of a foliar organ.

Pollen tubes.—The pollen tubes are very numerous and prominent. They penetrate the upper end of the ovary cavity and follow the ridge to which the ovules are attached (figs. 4, 5). They are so

intimately associated with the cells of the carpel in this region that it is frequently impossible to distinguish them (figs 6, 7). The tips of the pollen tubes along the edge of the ridge turn into the ovarian cavity (figs. 6, 7). The number of pollen tubes is so much in excess of the number of ovules that only one in many ever reaches an ovule, the others disintegrating (fig. 8). The entrance of the pollen tube through the micropyle and into the embryo sac is clearly visible, the remnant of the tube usually persisting for a considerable time after fertilization (figs. 10–13, 16.) In one case the pollen tube had formed three branches, only one finding its way into the embryo sac (fig. 12). It is very generally recognized that the pollen tube is parasitic on the ovary of the sporophyte, but it is not generally considered as parasitic on the female gametophyte. In this study one case was observed in which the pollen tube penetrated the embryo sac and destroyed its contents (fig. 9). This has previously been observed by the writer in the case of *Passiflora adenophylla*.[1] Why the pollen tube occasionally behaves in this manner the writer does not attempt to answer.

Nucellus.—The nucellus at first is composed of small cells which are very compact at time of fertilization (figs. 13, 25, 26). It increases very rapidly, the cells increasing both in number and size and becoming very vacuolate. Those cells lying next to the endosperm disintegrate very rapidly, so that there is never a large amount of nucellus at any one time (fig. 30). A small amount of nucellus persists in the region of the hilum after maturity.

Fertilization.—The disintegration of the antipodals and the enlargement of the embryo sac begin with or just before the entrance of the pollen tube into the sac (figs. 1, 3, 30). The polar nuclei unite at this time and apparently just before the entrance of the pollen tube (fig. 2). It is very doubtful whether triple fusion occurs in this species. The relationship of the entrance of the pollen tube into the embryo sac to the behavior of the synergids was not observed, but it is evident that both synergids disintegrate at about the time of the entrance of the tube (fig. 10). The actual union of the sperm nucleus and the ovum was not observed, but the tip of

[1] Cook, M. T., Notes on the embryo sac of *Passiflora adenophylla*. Bull. Torr. Bot. Club **36**:273–274. 1909.

the pollen tube just penetrating the sac was observed repeatedly (figs. 10–13, 16). In one case the tip of the tube contained two well defined bodies, although the embryo was well advanced (fig. 11). These two bodies had the appearance of two sperm nuclei. If they were sperm nuclei, the embryo was evidently forming parthenogenetically. On the other hand, these two bodies may have been fragments of a single disintegrating nucleus. In that case, one sperm nucleus may previously have united with the egg.

EMBRYO.—The development of the embryo, like that of many other of the legumes, is extremely variable. The first stage of development is in the form of a sphere (fig. 10), but the divisions may be very irregular (figs. 11, 12). In some cases the nuclei appear to divide well in advance of the formation of walls (fig. 11). In most cases the divisions are regular and result in the formation of a spherical or more often of an elongated embryo, which gives rise to the embryo proper and the suspensor (figs. 14–16). The suspensor is comparatively slow in its development. At first it is composed of a few large cells (fig. 15), but finally develops into a prominent organ bearing a much smaller embryo proper (fig. 16). It is extremely variable, in some cases being large and smooth (fig. 16), and in others covered with projections (figs. 17, 18). These projections are short, unicellular, trichome-like elongations from surface cells. This development of the embryo is very similar to that described by GUIGNARD[2] for *Spartium junceum*. He has also observed outgrowths on the suspensor of some species. Following this period of development, the embryo proper enlarges, and very early shows a differentiation leading to the development of the cotyledons (fig. 19). The cotyledons are well advanced; in fact the embryo has almost reached its complete growth before there is any evidence of cell division for the formation of the plumule (fig. 20). The radicle starts to develop a little later than the cotyledons (fig. 21). The suspensor persists until very late, but the embryo has undergone such enlargement that it is comparatively small at this time.

[2] GUIGNARD, M. L. Recherches d'embryogénie végétale comparée. Ann. Sci. Nat. Bot. **12**:5–166. 1881.

Polyembryony was observed in two cases (figs. 22, 23). In one case there were two and in the other four embryos. GUIGNARD observed cases of two and three embryos in the sacs of *Mimosa Denhartii*, and was of the opinion that the extra ones were derived from synergids. In the case of *C. sagittalis* the writer was unable to determine this point, but the early disintegration of the synergids makes it improbable that the extra embryos came from this source. They are most likely derived as a result of a splitting of the primary embryo derived from the egg.

ENDOSPERM.—The endosperm nucleus divides before the first division of the embryo and develops very rapidly. It forms a delicate lining for the entire embryo sac, with considerable masses immediately surrounding the embryo and at the opposite end of the sac (figs. 10–13, 16, 24, 26). The nuclei are abundant and never in more than one layer, except immediately surrounding the embryo and at the opposite end of the sac. The endosperm practically disappears with or just before the last of the nucellus cells and the inner cells of the seed coat (fig. 29).

SEED COAT.—A single seed coat is formed, which at first consists of four layers of cells except in the micropylar and hilum regions, where they are more abundant (figs. 13, 25, 26). These cells are thin and flat when young, but those of the outer layer soon tend to thicken and become more or less cubical (fig. 26). This tendency to thicken continues until the cells become columnar, the long axis being at right angles to the surface of the ovule (figs. 27–29). The cells of the second and third layers tend to become cubical, and the innermost layer of cells disintegrates (fig. 27). This is followed by a continued elongation of the cells of the outer layer, the formation of intercellular spaces between the cells of the second layer, and a disintegration of the cells of the third layer (figs. 28, 29). In the meantime the cuticle has become highly developed, and the walls of the cells of the two remaining layers stain as though highly cutinized. Fragments of the two inner layers of cells, of the nucellus, and of the endosperm may be seen at this time (fig. 29).

CLEAVAGE PLANE.—The formation of the cleavage plane in the hilum is very marked between two layers of cells which are richer

in protoplasm and stain more deeply than the surrounding cells (fig. 31). The cells of these two layers tend to become cubical and then columnar, the same as the outer layer of the seed coat (figs. 31–33). The line of demarcation becomes more and more conspicuous, and exposed surfaces of the two layers of cells become cutinized. Long before the seed is mature, the two layers can readily be separated by a slight pressure on the cover glass.

This work was done at the New York Botanical Garden during a period of leave of absence from Rutgers College.

RUTGERS COLLEGE
NEW BRUNSWICK, N.J.

EXPLANATION OF PLATES XXX, XXXI

FIG. 1.—Eight-celled embryo sac; antipodals disintegrating and polar nuclei uniting.

FIG. 2.—Eight-celled embryo sac; antipodals have disappeared and polar nuclei united.

FIG. 3.—Diagram of ovule with eight-celled embryo sac.

FIG. 4.—Diagram of inner dorsal side of ovary, showing ridge, points of attachment of ovules, and entrance of mass of pollen tubes (*pt*).

FIG. 5.—Cross-section of diagram of ovule, showing one ovule and dorsal ridge.

FIG. 6.—Upper part of ovary, showing entrance of pollen tubes.

FIG. 7.—Part of same under higher power.

FIG. 8.—Disintegrating pollen tubes that have failed to reach ovules.

FIG. 9.—Pollen tube destroying contents of embryo sac (semi-diagrammatic).

FIG. 10.—Very young embryo; tip of pollen tube attached; endosperm well advanced.

FIG. 11.—Embryo; prominent endosperm; tip of pollen tube showing two bodies.

FIG. 12.—Embryo; endosperm; tip of pollen tube which has branched.

FIG. 13.—Part of ovule, showing seed coat, nucellus, micropyle, embryo, and endosperm; *pt*, pollen tube.

FIG. 14.—Embryo.

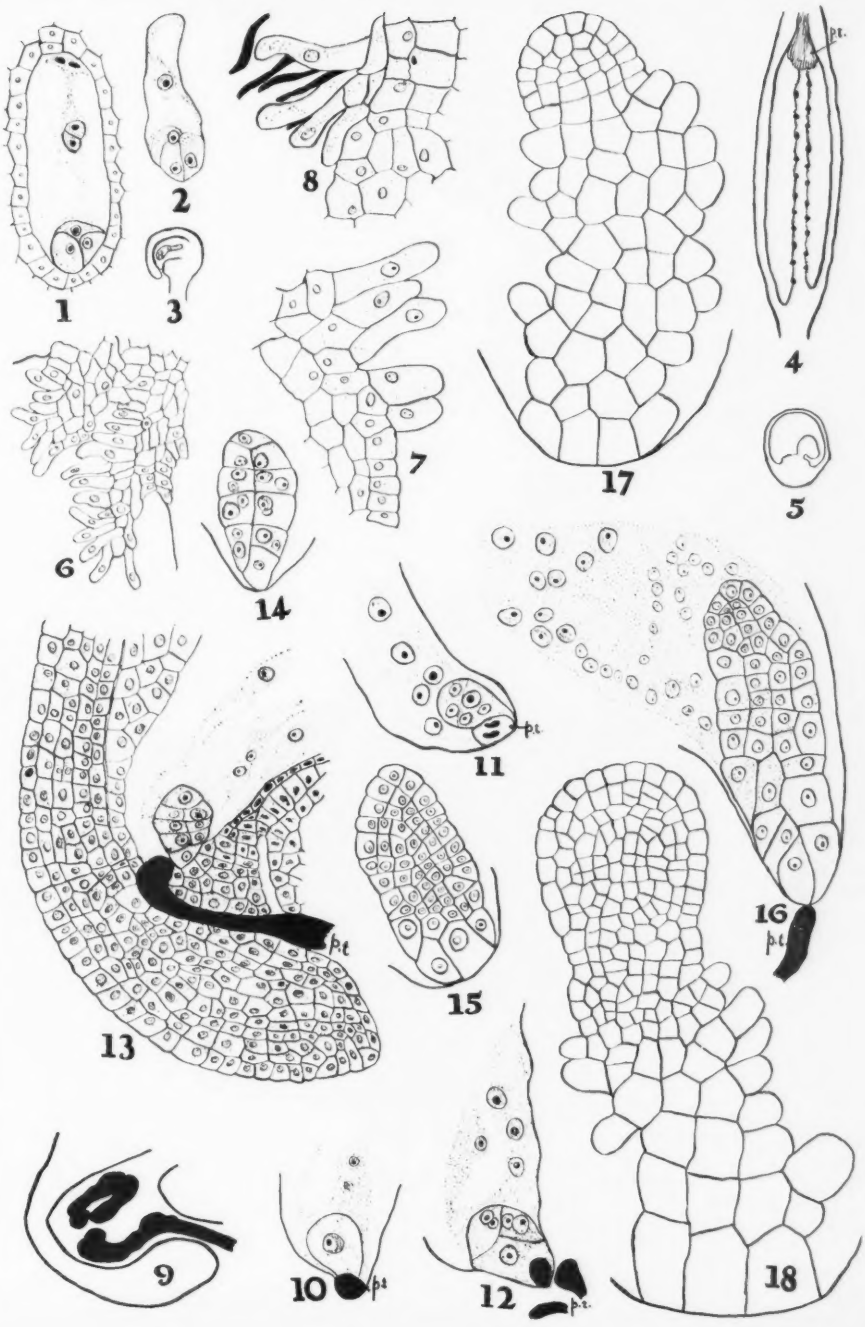
FIG. 15.—Embryo.

FIG. 16.—Embryo and endosperm; *pt*, pollen tube.

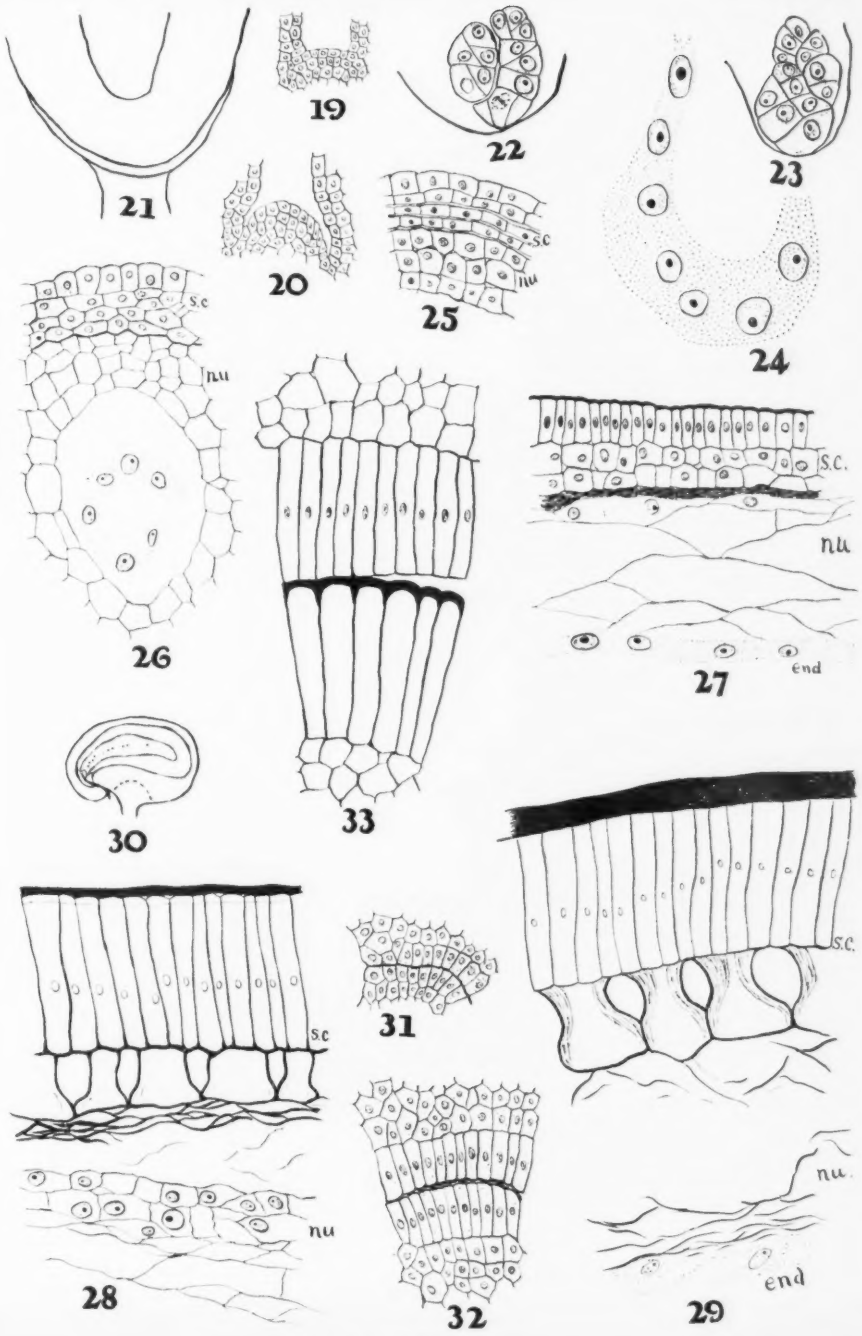
FIG. 17.—Embryo and prominent suspensor.

FIG. 18.—Embryo and prominent suspensor.

FIG. 19.—Part of embryo, showing origin of plumule.



COOK on CROTALARIA



COOK on CROTALARIA

FIG. 20.—Part of embryo, showing origin of plumule (older than fig. 19).

FIG. 21.—Part of embryo, showing origin of radicle (diagrammatic).

FIG. 22.—Polyembryony.

FIG. 23.—Polyembryony.

FIG. 24.—Endosperm in end of sac opposite embryo.

FIG. 25.—Part of ovule, showing four layers of cells of seed coat (*sc*), and three layers of cells of nucellus (*nu*).

FIG. 26.—Part of ovule, showing four layers of cells of seed coat, part of nucellus, and endosperm lining embryo sac; cut at right angles to fig. 25.

FIG. 27.—Three layers of cells of seed coat, fourth layer disintegrating; nucellus and endosperm.

FIG. 28.—Two layers of cells of seed coat, third layer disintegrating; nucellus disintegrating.

FIG. 29.—Two layers of cells of seed coat; disintegrating nucellus and endosperm.

FIG. 30.—Diagram of ovule about same age as fig. 13.

FIG. 31.—First stage in formation of cleavage plane.

FIG. 32.—Second stage in formation of cleavage plane.

FIG. 33.—Third stage in formation of cleavage plane.

CYTOLOGICAL AND PHYSIOLOGICAL CHANGES IN VICIA FABA IRRADIATED WITH RÖNTGEN RAYS[1]

HIDEO KOMURO[2]

(WITH ONE FIGURE)

In 1922 the writer published a preliminary note on the cells of *Vicia Faba* modified by Röntgen rays, and their resemblance to tumor cells.[3] Because of this resemblance, these experiments have been repeated, using different kinds of X-ray tubes and a race of *Vicia Faba* called "Wasesoramame," which was obtained from Nihon-Noen in Tokyo. Microscopical observations cannot be made on the material of the 1923 experiments in the near future, so that at this time the results of the cytological experiments of 1922 are reported briefly, with observations on the physiological aspects of the 1923 experiments. Methods will be explained in detail in a later complete paper.

The Röntgen tube used was OKURA'S gas tube with tungsten anticathode, the hardness of which was ± 10.5° of WEHNELT. Seedlings in water in a Petri dish were placed under the X-ray bulb, the distance between the anticathode and the material being about 30 cm., and were irradiated with a current of 2.0–2.5 milliampères for one hour.

Cytological observations

Beginning 1.5 hours after irradiation, degeneration phenomena were observed as follows.

Preparations made 1.5 hours after irradiation: Vacuolization of cytoplasm apparent; chromatolysis clearly evident, only nuclei with distinct membranes being present; all observed mitoses abnormal.

[1] Preliminary note on cytological experiments of 1922 and observations on physiological aspects of experiments of 1923.

[2] Traveling Fellow from the Department of Education of the Imperial Nipponese Government.

[3] Bot. Mag. Tokyo **36**: no. 424. 1922.

Preparations made 6 hours after irradiation: Nuclear membranes not visible, owing to degeneration; nuclear contents stained dilute grayish black by haematoxylin, with numerous small granules stained deeply with the same dye (feinkörnige dichte Hyperchromatose); pyknotic and karyolytic conditions frequent; abnormal binucleate cells present. These changes are not observed in the control preparations.

Preparations made 9 hours after irradiation: Vacuolization of cytoplasm manifest; karyolytic and pyknotic conditions prevalent; escaped nuclei very often seen in cytoplasm. There are many nuclei in the condition of "feinkörnige dichte Hyperchromatose" and of "Kernwandhyperchromatose" (chromatic substance adhered to the nuclear membrane), while a degeneration of the nuclear membrane and a deformation of the nucleus are of common occurrence. Many abnormal binucleate cells are found, as well as many others which appeared to be approaching this condition, for it seemed evident that the mitotic process was interfered with in the prophase stages, with the result that the nuclei had become constricted and irregularly divided. The process often bears a striking resemblance to amitosis. Many giant nuclei stained deeply by haematoxylin are found, and also multinucleolar nuclei. These alterations cannot be seen in the controls.

In the root tips of seedlings, these cytological changes occurred within 1.5 hours after exposure for one hour to Röntgen rays from a tungsten anticathode (gas tube). Nine hours after irradiation, binucleate cells, giant nuclei, and multinucleolar nuclei were found, and these changes resemble those described in the writer's preliminary note of 1922. Besides these conditions, various stages of degeneration were manifest, only abnormal mitotic figures being found 1.5 hours after irradiation.

Physiological observations

Before leaving Nippon the writer made five experiments for the purpose of examining the effect of soft rays from a molybdenum anticathode of a Coolidge tube. Tips of radicles of seedlings of *Vicia Faba* were preserved for the cytological investigation to be made after the physiological experiments were finished. The seeds

used for these experiments were of the "Wasesoramame" race. In the irradiation a water cell of aluminium was used.[4]

Experiment I

Two hours' irradiation of air-dried seeds (tube dist. ca. 15 cm.; 7°–8° (W.); 2d cur. 1.6–1.7 m.a.; temp. 16°–17° C.).[5]—Immediately after irradiation the seeds were sown in pots of sand and placed in a greenhouse. The treatment of seeds after irradiation was the same in all the experiments.

Seven days later ($162\frac{2}{3}$ hours after irradiation) the radicles of the irradiated seeds had reached a length of 2.2, 3.7, 3.8, 3.9 and 4.1 cm., and their tips were yellowish green. Fourteen days after irradiation, the length of the radicles varied between 4.5 and 7 cm., and some of them bore lateral roots. Two hours' irradiation from a molybdenum anticathode does not deprive the air-dried seeds of the power of germination.

Experiment II

Two hours' irradiation of seeds steeped 67 hours (2d cur. 1.6 m.a.; heat. cur. 4 amp.; 4.6°–7.4° C.).—The results are shown in table I, which shows the severe inhibitory effect of two hours' irradiation on the development of steeped seeds. The tips of radicles from the irradiated seeds were anomalous in form and in a somewhat necrotic condition.

Experiment III

Intermittent two hours' irradiation of seeds steeped 67 hours.—Seeds were exposed to rays for one hour under the same condition as in experiment II, and 45 hours later a secondary irradiation of one hour was made (4.6°–7° C.). The results are shown in table II. On the twelfth day after irradiation, the tips of the radicles became

[4] KOMURO, H., Studies in the effect of Röntgen rays upon the development of *Vicia Faba*. Jour. Coll. Agric. Imper. Univ. Tokyo **8**: 1923 (p. 284). The irradiation was made by the writer under the direct supervision of the late Professor K. FUJI at the Electrical Laboratory of the Agricultural Experiment Station of the Department of Agriculture and Commerce, Nishigahara, Tokyo.

[5] Abbreviations: tube dist., distance between anticathode of X-ray tube and seeds to be irradiated; 2d cur., secondary current; heat. cur., heating current; m.a., milliampères; amp., ampères; W., hardness of X-ray tube as shown by Wehnelt's measuring instrument; temp., temperature of place in which seeds were irradiated.

stumpy and harder than the controls, and blackish brown or black in color. Most of them were malformed and in a necrotic condition.

Experiment IV

Intermittent two hours' irradiation of seeds steeped 43 hours.— Seeds were exposed to rays for one hour (heat. cur. 4 amp.; 2d cur. 1.7 m.a.; tube dist. ca. 11 cm.; temp. 8.2°–10° C.), and $47\frac{2}{3}$ hours later they were again exposed for one hour under the same

TABLE I

Hours after irradiation	Control		X-rayed	
	Length (cm.)		Length (cm.)	
	Radicle	Plumule or shoot	Radicle	Plumule
$92\frac{1}{3}$	0.6–1.8	.....	0.6–1.0	.....
$162\frac{2}{3}$	5.5	4.2	2.0	3.7
	7.3	3.6	0.7	3.0
	4.5	4.3	0.3	Does not come out of seed coat
	9.1	5.2	.....	

TABLE II

No. of days after second irradiation	Control		X-rayed	
	Length (cm.)		Length (cm.)	
	Radicle	Plumule	Radicle	Plumule
5	4.3		0.7	
	3.9		0.7	
	1.3		.....	
10	Young plants having many lateral roots	Length above soil 3.5–5.0	1.3	1.0
			1.4	0.7
			2.3	1.0
			1.7	Does not come out of seed coat
			0.5	

conditions (temp. 6.8°–8° C.). In these experiments special care was taken to expose the seeds similarly to the rays, and, in the case of intermittent irradiation, to minimize the loss of water from the steeped and irradiated seeds.

Sixteen days after irradiation, X-rayed radicles had reached 0.3, 1.4 (three), 1.5, 1.6, 1.7 (two) cm. in length (average 1.36). They were stumpy, hard, blackish brown or black, and were in a necrotic condition, while the control plants developed to the stage having 3–5 nodes above the soil. Fig. 1 shows the state of growth sixteen days after irradiation, the tips of the radicles having been cut off for the purpose of cytological investigation.

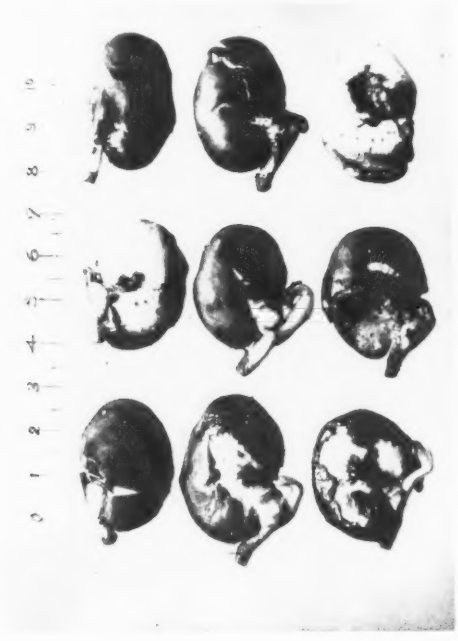


FIG. 1

Two hours intermittent irradiation produced more marked effects than did the continuous irradiation of the same duration, and the seeds thus treated ceased to grow at almost the same stage as is shown in fig. 1. Even in steeped seeds, two hours' irradiation could not deprive them of the power of germination. They could develop to a certain stage, however, verifying the writer's paper of 1923 (p. 287[1]).

Discussion

The writer has previously pointed out the resemblance of cytological changes caused by the irradiation with Röntgen rays to those appearing in tumor cells, and he has repeated the experiments on the effect of these rays, using the seedlings of another race of *Vicia Faba*, "Wasesoramame." As early as 1.5 hours after irradiation the degeneration process is under way, and nine hours after exposure one may observe various stages of degeneration. These resemble the conditions described in the writer's previous paper, and closely approximate those noted by him and other investigators in tumor cells. Although a cytological investigation of the material obtained this year could not be made, the physiological results were almost the same as in the formerly reported cases. This was especially true with regard to the effects of intermittent irradiation, which has never been employed by other investigators using plants. In the therapeutic use of Röntgen rays they were usually allowed to act on tissue intermittently. From these results it may safely be inferred that this intermittent use of X-rays in all probability will be harmful to the human body, although various filtering methods may be used; for X-rays may be summed up to harmful doses by repeating the exposure. Moreover, we must take the nature of the X-rays (that is, soft and hard) and the doses it is possible to give into consideration. The severe effect of rays from a tungsten anticathode (hard rays) at small milliamperages in cytological experiments should be borne in mind, and that of the physiological effects caused by soft rays from a molybdenum anticathode.

The practical bearing of the results of this investigation should be emphasized. The results of cytological and physiological experiments, together with the case of YAMAGIWA and ICHIKAWA'S artificial carcinom,[6] and the carcinom caused by the chronic stimulation,[7] and that of the Röntgencarcinom reported by BICHLER[8] have

[6] YAMAGIWA and ICHIKAWA, Mitt. d. Med. Fak. Kais. Univ. Tokyo. **15**:1915, **17**:1917, **19**:1918, **22**:1919. Amer. Jour. Can. Res. **11**:1917.

[7] BOVERI, TH., Zur Frage der Entstehung maligner Tumoren. 1914 (p. 37).

[8] BICHLER. Zur Kasuistik des Röntgencarcinoms. Wiener klin. Wochens. 1914. Nr. 20.

led the writer to the conclusion that the therapeutic use of X-rays will be dangerous and of questionable value, because they may be expected to cause cytological alterations resembling those in malignant tumor cells.

The writer is grateful to acknowledge his indebtness to the late Professor K. FUJI, who has kindly helped in making the exposures to rays, and to Professor L. W. SHARP, who has looked over the manuscript. Thanks are also due to the Morimura Hômei Kwai and to Mr. K. OHNISHI for defraying the expenses of the present research.

BOTANICAL LABORATORY
CORNELL UNIVERSITY

BRIEFER ARTICLES

GEORGE LINCOLN GOODALE

(WITH PORTRAIT)

Professor GOODALE was born August 3, 1839, at Saco, Maine. He graduated from Amherst in 1860, and received the degree of M.D. from Harvard in 1863. His first teaching position was at Bowdoin (1869–1872), where he was professor of "materia medica and natural science," a good illustration of the range of teaching demanded of a professor of science in those early days. In 1872 he became instructor in botany at Harvard; in the following year he was appointed assistant professor; and in 1878 became professor of botany, a position which he held until his retirement in 1909, when he became emeritus professor. He died April 12, 1923, in his 84th year.

Professor GOODALE was a notable teacher. It was the good fortune of the writer to work under him for two summers, during one of them as his laboratory assistant. He was certainly gifted as a remarkably clear and attractive lecturer, organizing and presenting his subject in a way that his pupils could never forget. As many students in those days remarked, he "really made botany interesting," in its early excursions beyond taxonomy. He was not only a great teacher, but also an unusually efficient organizer. Through his ability as a teacher and an organizer he was able to relieve Dr. ASA GRAY of the tedious routine of elementary instruction and many administrative cares. Associated with the abilities mentioned was a very kindly personality, so that his relations with students were very helpful and stimulating.

While his chief interest was in teaching and in building up a botanical equipment, his own field of work was in economic botany, in the improvement of useful plants and their products. In this connection, he was especially interested in the Harvard Experiment Station in Cuba.

Professor GOODALE will stand as probably the greatest teacher of botany in the early days, when botany was beginning to extend its range, and laboratory work began to be a feature. He was a pioneer in this extension, which has led to the great differentiation of the subject today.—J. M. C.

CURRENT LITERATURE

BOOK REVIEWS

The Cactaceae

The Carnegie Institution of Washington is to be congratulated on the completion of its monograph on the Cactaceae.[1] This large, important, and peculiar family of plants, almost wholly American, had long been in need of a thorough revision. The work was undertaken by N. L. BRITTON and J. N. ROSE. While the major part of the investigation was financed by the Carnegie Institution, considerable aid was given by the New York Botanical Garden, the United States National Museum, and the United States Department of Agriculture. The New York Botanical Garden gave the use of four greenhouses, financed several field trips, and prepared most of the paintings used for the colored plates. The Department of Agriculture furnished and equipped a large greenhouse, where at one time have been assembled about 5000 plants.

This investigation is undoubtedly the most comprehensive piece of cooperative work that has even been undertaken in any botanical field. The results have been epoch-making, and will for a long time remain the standard in this group of plants. The investigation has led, not only to the publication of four elaborate volumes, but to the assembling of two great cactus herbaria, one in the Smithsonian Institution and the other in the New York Botanical Garden. The collection in Washington occupies 48 standard cases with 1152 compartments. It contains representatives of all the genera and most of the species described in this work. The collection in the New York Botanical Garden, although perhaps not so large, possesses all the more important series.

The Carnegie Institution took up this project in 1912, but it is now twenty years since the authors began the study of the Cactaceae by field, greenhouse, and herbarium work. Perhaps no other group of plants presents so many difficulties to the systematic botanist as the Cactaceae, chiefly because it is very difficult to make herbarium specimens for preservation, and because the early students of this family preserved very few of their types. In other cases the types had been destroyed or lost. Thus many old descriptions had been incorrectly interpreted, plants had been wrongly identified, and such errors had been perpetuated. The authors, fully aware of this, made it the basis of their work to re-examine all the original descriptions.

As most of the Cactaceae had been first described by European botanists, ROSE went to study all the important herbaria and collections of living plants

[1] BRITTON, N. L., and ROSE, J. N., The Cactaceae. Descriptions and illustrations of plants of the Cactus family. 4to. 4 vols. 1919–1923.

in Europe in 1912, visiting England, France, Germany, Belgium, Switzerland, and Italy. Extensive field operations in the cactus regions of both Americas were undertaken by the authors and their assistants, and many naturalists have voluntarily helped to collect material. Thus in 1913, 1914, and 1915 the authors visited the West Indies; in 1914 Dr. and Mrs. ROSE went to Jamaica, Panama, Peru, Bolivia, and Chile; in 1915 ROSE explored the cactus regions of Brazil and Argentina; in 1916 those of Curacao and Venezuela; and in 1918 those of Ecuador. Dr. and Mrs. BRITTON have repeatedly visited the West Indies, and SHAFER collected for six months in the desert regions of Uruguay, Paraguay, Argentina, and Bolivia. All the cactus deserts of Mexico and the southern and southwestern United States had been widely explored before 1913 in repeated excursions by ROSE. No other study of the Cactaceae has been based on such an extensive and comprehensive collection of living and dried material, accompanied by notes and photographs made in the native haunts of these remarkable plants.

The last monograph of the Cactaceae was published by SCHUMANN in 1898, to which he added a supplement in 1903. His systematic disposition of the cacti was, with some alterations, chiefly that of SALM-DYCK of 1849, who in his day had been the great authority on this family. This classification was in many cases based on external vegetative characters, as flowers and fruits of most of the plants were then unknown. Efforts to use characters taken chiefly from the reproductive organs were first made by ENGELMANN, LEMAIRE, WEBER, and the writer of this review, but none of them had as rich material or as vast field knowledge of the entire family at their command as had BRITTON and ROSE.

The result of this comprehensive study is now presented in four large quarto volumes, abundantly illustrated by colored plates, photographs, and drawings.

In 1903 SCHUMANN recognized about 800 species, which he grouped under 20 very heterogeneous genera; while BRITTON and ROSE describe 1255 species under 124 genera. The number of genera may seem to be too large, and one might feel inclined to unite two or three here and there, yet the number would probably not be diminished, since one might also segregate a new genus from some of those described. As a rule, the genera are founded on characters of flowers and fruit, with which the vegetative characters and the geographical distribution agree. In fact, I regard the establishment of these new genera as the most important contribution, since they show in a very concise way the development which the family exhibits.

The authors maintain the three principal tribes already recognized by former writers: PERESKIEAE, OPUNTIEAE, and CEREAE. The first volume treats of the first two tribes. The tribe PERESKIEAE contains but one genus, *Pereskia*, with 19 species. These plants look like ordinary trees or shrubs with large flat leaves, rather than cacti, for the spines are more or less concealed. The tribe OPUNTIEAE is much more varied, having seven genera of various

habit, some resembling *Pereskia*, with well formed flat leaves. The bulk of this tribe is included in the genus *Opuntia*, which comprises 263 species, or 117 more than were known to SCHUMANN. It is the largest genus of the family, covering its whole range, and extending farther than any other genus both north and south toward the colder regions. Many are native to the United States, some extending as far north as British Columbia in the west, and Nantucket, Massachusetts, in the east. In its development this genus shows a marked uniformity in its reproductive organs, but a wide range in its vegetative characters. It has always been the stumbling block to the cactus student, who will therefore greatly appreciate this clear treatment, which will help to overcome difficulties in identifying these plants.

Volumes II–IV present the tribe CEREAE, which comprises the greatest variety of genera and species, and is divided into eight natural subtribes.

Volume II contains the subtribes CEREANAE and HYLOCEREANAE, which were formerly included in the unnatural and bulky genera *Cereus* and *Pilocereus*. The authors divide CEREANAE into 38 smaller and more natural genera. In this group are the giants of the family, the huge *Cereus peruvianus* and its allies; the strange genus *Cephalocereus*, of which *C. senilis*, covered with long curled white hairs, is popularly known in Mexico as the "old-man cactus"; the gigantic *Pachycereus Pringlei* from Sonora and Lower California, which reaches 11 m. in height; the even taller *Pachycereus chrysomallus* and *P. columna-trajani* from Puebla and Oaxaca, Mexico; and the giant cactus of Arizona and southeastern California, the *Carnegia gigantea*.

The subtribe HYLOCEREANAE includes all the vinelike species with trailing, climbing, or pendent branches, formerly in *Cereus*. They invariably emit aerial roots on the sides of their joints. Some of them are rampant high climbers, and many of them produce the finest and largest flowers among plants. They fall into nine very natural genera, of which *Hylocereus* and *Selenicereus* are both night-blooming and have the most showy and very fragrant flowers. *S. grandiflorus* is popularly known as "queen-of-the-night."

Volume III deals with the subtribes ECHINOCEREANAE, ECHINOCACTANAE, and CACTANAE, which contain most of the smaller forms, many of which are one-jointed. The first subtribe includes the North American genus *Echinocereus*, which extends from Mexico into the western United States, and as far north as Wyoming and Utah. They are all low plants, simple or branched at base, mostly with beautiful flowers, which easily grow and flower in cultivation as pot plants. BRITTON and ROSE describe several new species, so that the genus now numbers 60 species, whereas SCHUMANN had only 38 species. The other genera of this subtribe are all South American, usually with globular bodies, often growing in clumps, and in many cases having beautiful and highly colored flowers. They were included by former authors under various incongruous genera, while the new genera here proposed are most natural and concise. Some of these genera, particularly *Echinopsis*, have been known in cultivation, while the little red-flowered *Chamaecereus silvestri* and the little round-bodied *Rebutia minuscula* are recent introductions from Argentina.

The subtribe ECHINOCACTANAE is divided into 28 small genera, mostly made at the expense of the old heterogeneous genus *Echinocactus*. SALM-DYCK was dissatisfied with this genus, but despite several attempts made later to modify it, SCHUMANN still retained it. It is impossible in a short review to enter further into the details of these 28 genera. Suffice it to say that they seem well founded, although I would have divided *Ariocarpus* into two, and I regret that the rules of nomenclature obliged the authors to employ such an ugly sesquipedalian name as *Echinofossulocactus*, for which SCHUMANN'S subgeneric name *Stenocactus* would be preferable if allowable. Some of the giants of this subtribe are retained under *Echinocactus* as established by LINK and OTTO in 1827; here belong *E. grandis*, *E. ingens*, and *E. visnaga*, 50–100 cm. in diameter, and often 1–3 m. high.

The dwarfs of this subtribe are *Epithelantha micromeris* from western Texas and northern Mexico, and the eight species of *Frailea*, all natives of South America, some of them only 1 cm. in diameter. Of great biological interest is *Lophophora Williamsii*, which during its resting period sinks almost entirely into the ground and hides itself under the dust until the rainy season sets in, swelling it up and washing it clean, so that it reappears above ground and opens its small pink flowers. It is devoid of spines. Of great interest also are the representatives of *Ariocarpus*, with their horny and in some species fissured epidermis, which makes them look much like the surrounding stones where they grow, and which often nearly cover them. *A. fissuratus* is said to be easily overlooked among the rocks when not in flower. Some of the species are often called rock cactus. This is a case of parallelism shown in the mimicry of a number of species of *Mesembrianthemum* in South Africa.

Of the subtribe CACTACEAE, *Cactus*, or *Melocactus* as it was commonly called by former authors, is popularly known as "Turk's cap," alluding to the cylindrical flower and fruit bearing cephalium on the top of the plant body. These species, which are very common in the West Indies, are extremely variable, and an almost incredible number of so-called species had been described, but happily the authors, after a careful study in the field, chiefly by BRITTON, were able to reduce the number of real species to 18. They range from Brazil to the West Indies on the east, and from Peru through Central America to southern Mexico on the west.

Volume IV concludes the tribe CEREAE with the last three subtribes. The subtribe CORYPHANTHANAE contains 14 genera, and is built up partly from the *Echinocactus* and *Mammillaria* of older authors. These plants, with a few exceptions, have globular bodies, and small but brightly colored flowers, and many of them are favorites among amateur cactus growers. Unfortunately, because the old familiar name *Mammillaria* is a homonym of a genus of Algae, it had to be changed. It is named *Neomammillaria*. Despite the fact that a large number of species were taken out of the old genus *Mammillaria*, of which SCHUMANN knew 105, so many new species have been added that in this much more restricted sense BRITTON and ROSE recognize 150, the bulk of which (122) are native of Mexico.

The subtribe EPIPHYLLANAE contains mostly epiphytic plants, often with flat, leaflike, and spineless joints, and some with very showy flowers, of which *Zygocactus* and *Epiphyllum* (the *Phyllocactus* of SCHUMANN and other authors) are much grown stove plants. The critical treatment of this subtribe is especially interesting, and many new facts are recorded.

The eighth and last subtribe, RHIPSALIDANAE, is divided into 8 genera mostly new or revived. They are almost all graceful epiphytes, with small and usually white flowers. The student of the very difficult genus *Rhipsalis* will be glad to see almost every species illustrated.

An extended appendix enumerates a great amount of additional data, including a number of new species, and even two new genera, which were discovered while the volumes were passing through the press. A very careful and complete index covers 28 pages, with four columns to the page, and contains about 10,000 plant names.

Except for ENGELMANN'S classical publications on the Cactaceae, no comprehensive study of this family, almost exclusively American in its origin, had come from America. The Carnegie Institution and the authors deserve great credit and the thanks of botanists for presenting such a full and attractive account of these remarkable plants.—A. BERGER.

Age and area

Among the theories propounded during the past two decades to account for the facts of plant distribution, none has attracted more attention than the "age and area" hypothesis of WILLIS. Formulated in 1915, it has been elaborated in a series of publications appearing from time to time as the author's studies have progressed and new data have been accumulated. This journal has noted the gradual development of the theory,[2] and now welcomes a volume in which the substance of previous articles is brought together in organized and concise form.[3]

WILLIS states the theory as: "The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climates from one region to the next, or other ecological boundaries, and the like, also by the action of man, and by other causes."

The present position of the theory is discussed in the first part of the book, particularly in a chapter contributed by GUPPY. Among the points emphasized as fundamental are the origin of each species in one limited area, and its migra-

[2] Rev. in BOT. GAZ. **61**:82–83. 1916; **62**:160–161. 1916; **63**:419–420. 1917; **65**:116–117. 1918; **65**:486. 1918; **70**:324–325. 1920.

[3] WILLIS, J. C., Age and area: a study in geographical distribution and origin of species. 8vo. pp. vii+259. London: Cambridge Univ. Press. 1922.

tion from the small area to a larger; the relative slowness of this migration and the general similarity of the results under somewhat similar conditions; the rapid spread of introduced species usually due to the changes of conditions that have been made by man; and the fact that acclimatization under natural conditions extends over vast periods of time with very small steps leading to vastly different conditions.

Recognizing these conditions, it is shown that predictions based on the hypothesis have been verified. It is also evident that most of the objections that have been urged against the theory are due to misunderstandings of its terms or to the neglect of some of its qualifications. One of the most common errors has been to show that it fails when applied to one or two species, while the author distinctly states that it is to be applied to groups of ten or more.

The second part of the volume opens with an extension of the main hypothesis under the name "size and space." This may be expressed as "on the whole, keeping within the same circle of affinity, a group of large genera will occupy more space than a group of small. The space occupied will vary more or less with the number of species." In a chapter by SMALL it is shown that in the Compositae the average generic area and the average number of species per genus vary together, and are closely related to absolute age. Mrs. REID contributes a chapter in which palaeobotanical evidence is advanced which is favorable to the age and area hypothesis, and in another DEVRIES shows that it harmonizes perfectly with and indeed depends upon the origin of species by mutation.

Other topics as indicated by chapter headings are endemism and distribution, monotypic genera and genera of larger size, the hollow curve of distribution, the applicability of age and area to animals, and the origin of species.

In a more recent paper[4] are replies to certain criticisms that have been made, the principal ones being based on the failure of the critics to realize that the theory cannot be applied to less than ten allied species at once, and the view that endemic forms are chiefly relics. In reply WILLIS asserts that although relic endemics are common, they are quite lost in the crowd of initials. It is shown that the nearer an island is to the mainland, the larger the proportion of monotypes among its endemics, a phenomena which is difficult to explain if endemics are mostly relics. In spite of the logical nature of these replies, it seems to the reviewer that too little weight has been given to relic endemism, and insufficient account taken of the dying out of species. This, however, would not invalidate the theory as a whole, nor prevent its application to most regions of the world. WILLIS is certainly to be commended for the vast quantity of data upon which his hypothesis has been founded, and for its stimulating effects upon studies of geographical distribution.—GEO. D. FULLER.

[4] WILLIS, J. C., Age and area: a reply to criticism with further evidence. Ann. Botany 37:193–216. 1923.

The pharmacists' botany

Botany is a many sided science, and many textbooks have been written attempting to meet the need of students of plant life. One group of students, however, has been neglected hitherto, but their needs are now met by RIGG,[5] who has prepared a textbook of botany for the students of pharmacy. The book reminds us that once botany was mainly a study of drug plants, and that physicians were the chief botanists.

The book is very readable, but quite elementary, testifying to the grade of requirements demanded of pharmacists so far as botany is concerned. It might very well be used with high school students, except for the scientific nomenclature of drug plants. From much of the text one feels that after all what is wanted is a knowledge of the same facts about plants that the student obtains from any other good elementary textbook of botany, but it gets its pharmaceutical flavor by having the examples and illustrations taken from the list of drug plants in the U.S.P., and by a very brief paragraph at the end of each chapter, bearing some such title as "some pharmaceutical buds," "some pharmaceutical leaves," etc., so that the student will know that he is really getting something that will help him in pharmacy.

The text is organized into three parts. Part I is devoted to seed plants, with chapters on organs, the cell, plant names, leaves, stems, buds, roots, physiological processes, flowers, seeds, fruits, life histories, and the classification of seed plants. The last of these chapters is unique in consisting mainly of a list of about thirty-four pages of official and unofficial drugs from the various families of seed plants. Part II deals with spore plants, and contains chapters on thallophytes, bryophytes, and pteridophytes. Part III, on some other phases of pharmacy botany, considers ecology, propagation, and breeding as subjects contributory to the growing of drug plants.

The appendices present an outline of the plant kingdom, and a set of botanical synonyms of the common names used. An unusually full index enables one to locate all the references to each plant mentioned in the text. As the author has had fourteen years of successful experience in teaching botany to pharmacy students, the book should commend itself to others who are engaged in similar work. Students will find it entertaining and instructive, and the text surely does not demand too much of them.—C. A. SHULL.

MINOR NOTICES

Vegetation maps.—The lack of adequate and accurate maps of the vegetation of the world has been appreciated and deplored by all ecologists and plant geographers. These workers will therefore welcome certain recent additions as indicative of actual progress. GOODE'S[6] *School Atlas* contains a number of

[5] RIGG, G. B., The pharmacists' botany. 8vo. pp. xvii+303. New York: Macmillan Co. 1924.

[6] GOODE, J. P., School atlas; physical, political, and economic. pp. xii+41, 96 plates of maps. Chicago and New York: Rand McNally and Co., 1923.

such maps, including one of the vegetation of the world after SCHIMPER. It is a decided improvement on the original, and if the coastal strip of rain forest in eastern Africa could be transferred to the corresponding coast of South America, it might be regarded as quite satisfactory. In the same publication is a map of the vegetation of the United States and Canada. The plant covering of the former country is accurately and carefully delimited by SHANTZ and ZON of the United States Department of Agriculture, but the utility of the entire map is almost destroyed by the errors in the Canadian portion. A long strip of forest characterized by chestnut and chestnut-oak extends from Toronto and along both sides of the St. Lawrence River to the Gaspé peninsula. This is very misleading, for there is certainly not a tree of either species to be found in the Province of Quebec. Then the coincidence between the boundary line which separates Maine and New Brunswick and that which divides two forest formations is as absurd as it is erroneous. The two countries are parts of one physiographic and phytogeographic unit, artificially separated by an international boundary line determined by political compromise.

Perhaps the most important of the recent phytogeographic maps is one of Africa, prepared by SHANTZ[7] for the use of the "American Commission to Negotiate Peace." The wealth of material reviewed and digested in producing this map and the accompanying descriptive text is indicated by a bibliography of 400 citations. The vegetation is classified along ecological lines into twenty-one types or formations, among which tropical rain forest, various savannas, and deserts are most conspicuous. These formations are represented on the map in a manner that results in easy legibility. Accompanying the vegetation map are others of rainfall distribution, soil, and land classification.

A minor contribution has been made to our knowledge of the vegetation of South America in a map of Peru by WEBERBAUER,[8] on which the distribution of twenty-five association types is delimited. The accompanying text has brief descriptions of these types.—GEO D. FULLER.

Determination of hydrogen ions.—The second edition of this excellent treatise[9] reflects in part the rapid advances made in hydrogen ion concentration studies during the several years since the appearance of the first edition. The book has been revised where necessary, and some slight changes of organization have been made. Thus the first and eleventh chapters have each been divided into two, increasing the total number of chapters to twenty-one. The chapter on the choice of indicators has been much enlarged, and the chapters dealing with

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[7] SHANTZ, H. L., and MARBUT, C. F., The vegetation and soils of Africa. pp. 263. *pls. 2. figs. 50.* New York: Amer. Geogr. Soc. Research Series no. 13. 1923. $5.00.

[8] WEBERBAUER, AUGUST, Die Vegetationskarte der peruanschen Anden zwischen 5° und 17° S. Petermanns Geogr. Mitteil. **68**:89–91, 120–122. map. 1922.

[9] CLARK, W. M., The determination of hydrogen ions. 8vo. pp. 480. Baltimore: Williams & Wilkins. 1923.

approximate determinations of hydrogen ion concentration, hydrogen electrodes, and calomel electrodes are somewhat more extensive. Two tables have been added to the appendix, giving the standard values for calomel electrodes, and the relation of H+ to P_H. The literature citations occupy about forty pages in this edition.

The book is well made, and the author and publishers are making a valuable contribution to the advancement of research by keeping this invaluable manual up-to-date.—C. A. SHULL.

NOTES FOR STUDENTS

The primitive spindle.—BOWER[10] has published his conception of the significance of the "primitive spindle" in the embryology of the plant kingdom, unifying all the groups, from algae to seed plants. According to this view, the first cleavage of the zygote defines the polarity of the embryo, always being at right angles to the axis connecting the two poles. This cleavage results in "a spindle-like structure, and the term primitive spindle may be applied to it." It is stated that "comparison of plants in the embryonic state shows that a filamentous or spindle-like structure, with polarity defined by the very first embryonic cleavage, is common for them all." The relations to the primitive spindle of the various organs of the different groups are given in detail. For example, "the first root of all Pteridophytes that have a suspensor is clearly lateral. It projects from the side of the primitive spindle, and is not itself a part of it." In this way the different organs are interpreted, and the embryology throughout the whole plant kingdom referred to one fundamental organization.—J. M. C.

Fertilization in cotton.—KEARNEY[11] has investigated the problem of crossing and selfing in cotton, especially with the Pima variety of Egyptian cotton, although comparisons with upland cotton are included. The experiments were conducted at Sacaton, at the Pima Indian Agency in southern Arizona, and extended through eight years. He finds that, although the cotton flower is well adapted to cross pollination, most of the ovules are usually self-fertilized, the percentage of hybrids produced when two distinct varieties are grown side by side being small. He also investigated the structure and later ontogeny of the flower, the deposition of self pollen and foreign pollen upon the stigmas, and the competition of the two kinds of pollen. The bulletin is an interesting addition to the literature of pollination.—J. M. C.

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[10] BOWER, F. O., The primitive spindle as a fundamental feature in the embryology of plants. Proc. Roy. Soc. Edinburgh **43**:1–36. *figs. 26.* 1922.

[11] KEARNEY, T. H., Self-fertilization and cross-fertilization in Pima cotton. U.S. Dept. Agric. Bull. 1134. pp. 68. 1923.

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Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italic*.

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